BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

EDITORIAL BOARD

Managing Editor, PHILIP F. WAGLEY

E. COWLES ANDRUS
CHARLES R. AUSTRIAN
HENRY T. BAHNSON
KENNETH C. BLANCHARD
JOHN EAGER HOWARD
ARNOLD R. RICH

VOLUME XCIV

BALTIMORE
THE JOHNS HOPKINS PRESS
1954

Hospital Library

Copyright, 1954
by The Johns Hopkins Press

DOES NOT CIRCULATE

VOLUME 94 NUMBER 1

Comp.

UNIVERSITY OF MICHIGAN

FEB 8 1954

MEDICAL LIBRARY

Hosp.

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

January, 1954

In Vivo Studies of the Frog's Liver during the Excretion of Thioflavine S and Vasoflavine. ALLAN L. GRAFFLIN AND ELLEN G. CORDDRY 1

In Vivo Studies of the Frog's Liver during the Excretion of Esculin. ALLAN L. GRAFFLIN AND ELLEN G. CORDDRY 22

The Aperiodic Ballistocardiograph. S. A. TALBOT, D. C. DEUCHAR, F. W. DAVIS, JR. AND W. R. SCARBOROUGH 27

Observations on the Etiology of Retrolental Fibroplasia. HARRY H. GORDON, LULA LUBCHENCO AND IVAN HIX 34

Book Reviews 45

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

COPYRIGHT, 1954, BY THE JOHNS HOPKINS PRESS

BULLETIN OF

THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, PHILIP F. WAGLEY

ASSOCIATE MANAGING EDITOR, VERNON B. MOUNTCASTLE

EDITORIAL BOARD

E. Cowles Andrus — Charles R. Austrian — Henry T. Bahnson

Kenneth C. Blanchard — John Eager Howard

Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

Subscription price. In the United States and its possessions $8.00 per year. In Canada $8.25 per year; in foreign countries $8.50 per year. Single numbers, $1.00.

New subscriptions and renewals are entered to begin with the first issue of the current year. Subscribers in the United States are hereby notified that missing numbers cannot be replaced unless notification of loss is received in this office within 1 month following publication of the missing number; foreign subscribers 6 months.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

Manuscripts. Manuscripts should be typewritten, one side of page only, and double-spaced. Following the title should appear the author's name, department, and the university and/or hospital he represents. A list of references (bibliography) in numerical sequence should appear at the end of article. Preferred style for references is as follows: Author's last name, initials. Title of book or article, with first word and proper names capitalized only. Name of journal (abbreviate), date of publication, volume number: page number. The Bulletin is not responsible for loss of a manuscript in mail. Author should retain a duplicate copy.

Illustrations and Tables. Legends are required for Figures and descriptive headings for Tables. Use Arabic numerals to number Figures (Fig. 1); use Roman numerals to number Plates and Tables (Plate I). It should be indicated exactly where Figures and Tables are to appear in text; Plates all appear at end of article. All Figures should be drafted in black India ink for 50% reduction to 4½ inch width. Glossy photographs must be supplied for all Plates. Original illustrations will be returned to author with galley proofs.

Reprints. Twenty-five reprints, without covers, are furnished gratis for each article. Additional reprints should be ordered when galley proofs are returned.

THE JOHNS HOPKINS PRESS

BALTIMORE-18, MARYLAND, U. S. A.

Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

IN VIVO STUDIES OF THE FROG'S LIVER DURING THE EXCRETION OF THIOFLAVINE S AND VASOFLAVINE*

ALLAN L. GRAFFLIN AND ELLEN G. CORDDRY

Department of Anatomy, The Johns Hopkins University School of Medicine

Received for publication October 26, 1953

Beginning with the pioneer studies of Ellinger and Hirt (1, 2), fluorescence microscopy of the liver—following the infection of fluorescent dyes or of the glycoside, esculin—has been used by a number of investigators for direct visualization, in living and freshly sacrificed animals, of the bile canaliculi and other aspects of hepatic structure and function. The more important references to the literature have been included in recent publications (3–7), and need not be repeated here. Fluorescein, in the form of its sodium salt (uranine), has been most extensively employed; and the sequence of events during its excretion, under normal conditions, has been reasonably well established for both amphibia and mammals.

Among the other fluorescent dyes, thioflavine S has been found to give some of the most highly informative pictures of the bile canalicular system. A certain amount of detailed information is available upon its hepatic excretion in mammals, but the data for amphibia are more or less fragmentary (3). It is this dye, in both its conventional and its more purified form (vasoflavine), and particularly as observed *in vivo* during its excretion by the frog's liver, which is the subject of the present report. Thioflavine S was first used to any extent for intravital studies (primarily of the frog's kidney) by Singer (8, 9), who emphasized its marked photosensitivity and described striking photodynamic effects upon the renal glomeruli. He briefly mentioned similar effects in the liver, but stated (8) that "it did not seem to affect the color or appearance of the tubuli or their contents." The latter statement has not been borne out by subsequent experience; the fluorescent images of the canaliculi ("tubuli"), while initially quite sharp and exquisite, are rather rapidly extinguished upon exposure to ultraviolet light (3).

Of particular importance is Schlegel's (10) introduction of thioflavine S as a selective stain for blood vessels actively functioning at the time of injection. The success of the method depends upon a marked yellow-green fluorescence imparted to the vessel walls, in contrast to the predominantly blue fluorescence which appears in the hepatic cells and bile canaliculi. Schlegel's initial and sub-

* The "Ultropak" microscope and accessories employed in this study were made available on loan from the Armed Forces Institute of Pathology. The investigation was supported in part by a grant from the Damon Runyon Memorial Fund. We are deeply indebted to Miss Virginia V. Croker for patient secretarial assistance with the protocols, and to Miss Nancy M. Gray for technical assistance.

sequent studies (11, 12) led to the development, by Moses, Emery and Schlegel (13), of a purified fraction of the dye which they named vasoflavine, and which they regarded as almost ideal for the visualization of blood vessels under ultraviolet light. Moses *et al.* concluded that vasoflavine probably exists in aqueous solution as an equilibrium between monomeric and various polymeric forms, the proportion of the latter increasing with increasing concentration of the solution; that the yellow-green fluorescence is apparently produced by the polymeric forms and the bluish fluorescence by the monomeric form; and that the polymers are probably too large in molecular size to pass readily the barrier of the vessel walls, while the monomeric form quickly diffuses out of the vessels, thus explaining the yellow-green fluorescence of the vessel walls and the bluish fluorescence of the surrounding tissue. In exploratory studies they found no apparent toxic effects in mammals with blood concentration as high as 275 mg. %.

Vasoflavine has been used with excellent results by Moses and Schlegel (14), in a study of the juxtamedullary circulation of the kidney in rabbits; and in this laboratory (5) for delineation of the bile canaliculi in mice subjected to extrahepatic biliary obstruction. Also, some observations have been reported upon various abnormalities of the liver which can be visualized by fluorescence microscopy following injection of the dye (6). Since it was regarded as desirable to obtain more detailed information upon the hepatic excretion of thioflavine S by the amphibian liver, it was decided to investigate at the same time the elimination of vasoflavine, with a view to detecting possible differences in the behavior of the two samples of dye. As a supplement to the studies of the frog's liver, a limited series of observations has been accomplished upon the hepatic excretion of vasoflavine in the mouse, for comparison with data published earlier (3, 4) upon the elimination of thioflavine S in that species.

MATERIAL AND METHODS

The characteristics of the fluorescence microscope employed have been described elsewhere (3, 6). All observations were made with the 6× monocular and the 55× water immersion objective (without immersion cap and without the application of a cover slip to the surface of the liver). Male specimens of *Rana pipiens*, obtained at intervals from Vermont and after arrival maintained unfed in a standard ranarium, were used throughout. The studies were accomplished during the months of June and July. All experiments were conducted in a room controlled at c. 22° C., and frogs were routinely brought to the room ahead of time (usually overnight) for pre-conditioning. In the laboratory, they were placed in shallow water in individual glass jars, the water being changed at frequent intervals except in experiments of short duration. Frogs were pithed (brain and cord) and covered with water-soaked cotton; the liver was then promptly exposed by procedures described elsewhere (15), with scrupulous avoidance of its surface. The ventral surfaces of the left upper and left lower lobes (in that order) were routinely studied in turn. Each lobe was subjected to a continuous, sweeping survey, critical observations being limited to 2 minutes per lobe. It is these initial findings which are summarized in the accompanying tables, for which the following pertinent

information is supplied: *Weight of frogs*—Table I, range 19.0–28.3, average 23.6 gm.; Table II, 19.3–28.9, 24.2; Table III, 17.8–25.7, 21.7; Table IV, 17.6–31.1, 23.9. *Delay from initiation of pithing to first visualization of liver (left upper lobe)*—Table I, range 75–130, average 97 seconds; Table II, 80–115, 93; Table III, 70–120, 89; Table IV, 55–120, 98. *Delay from completion of observations on left upper lobe to first visualization of left lower lobe*—Table I, 15–60, average 28 seconds; Table II, 10–75, 25; Table III, 5–35, 17; Table IV, 10–40, 28.

Dye solutions were freshly prepared before use in balanced saline (formula of Holtfreter (16) for frogs, mammalian Ringer's for mice). They were injected into the dorsal lymph sac in frogs and intraperitoneally in mice, always in a concentration of 1:1,000 except in frogs B123–B134 (Table IV). In these animals the 1:1,000 solutions were serially diluted, each time to half-strength, to concentrations of from 1:2,000 (for 15 mg. per kg.) to 1:64,000 (for 0.47 mg. per kg.). In this way, each frog received the dye injection in a volume of fluid equivalent to that required of a 1:1,000 solution for a dosage of 30 mg. per kg. This procedure was adopted to facilitate absorption of the dye from the dorsal lymph sac under comparable conditions. Thioflavine S (Colour Index (17) no. 816, lot no. 7), and the related dyes thioflavine T (C.I. no. 815, lot no. 1) and primuline (C.I. no. 812, lot no. 5), were obtained from Hartman-Leddon Co. Vasoflavine (also designated as Erie Flavine S Purif., lot no. 13763) and Erie Flavine S Techn. (their trade name for thioflavine S, lot no. 14289) were obtained from National Aniline Division, Allied Chemical & Dye Corp.

For the studies summarized in Table V, male specimens of the white mouse (weight range 20.0–25.8, average 23.6 gm.) were obtained from Carworth Farms (strain CF1), and were maintained on a diet of Purina Laboratory Chow. They were deprived of food following injection, but mice B16–B27 (studied from 3 to 30 hours later) were allowed water *ad. lib.* The mice were sacrificed by crushing the cervical cord, and the liver was promptly exposed and supported upon a flat plate, rigidly secured to the animal tray. Unless otherwise specified, all observations were made upon the ventral surface of the left lower lobe, and were limited to the first 2 minutes following initial visualization. When additional lobes were studied, observations were likewise limited to 2 minutes per lobe. The delay from time of sacrifice to initial visualization varied between 80 and 130 seconds, average 111 seconds. In mice B20–B27, the delay between lobes varied from 17 to 25 seconds. Upon completion of the observations upon the liver, all animals were carefully examined under ultraviolet light to detect any possible miscarriage of the intraperitoneal injection; none was found.

Absorption spectra (Fig. 1) were accomplished with the Beckman spectrophotometer (in silica cuvettes with 1 cm. light path), using the same samples of dye specified above. All solutions were freshly prepared in water which was first demineralized with conventional equipment, then distilled from alkaline permanganate in all-glass apparatus.

OBSERVATIONS

As a basis for understanding the observations to be reported, a knowledge is required of certain features of hepatic structure and function, as these are revealed by fluorescence microscopy. With this technic, penetration into the substance of the organ is rather sharply limited; and one derives his information from the fluorescent images at and just below the surface of the liver.

In the frog, in this region, the liver characteristically exhibits pictures of fairly simple and repetitive alternation of sinusoids and laminae, the latter being typically two hepatic cells in thickness. Under our conditions, particularly at the rather high magnification (330×) routinely employed, the natural fluorescence of the laminae is of low or very low intensity

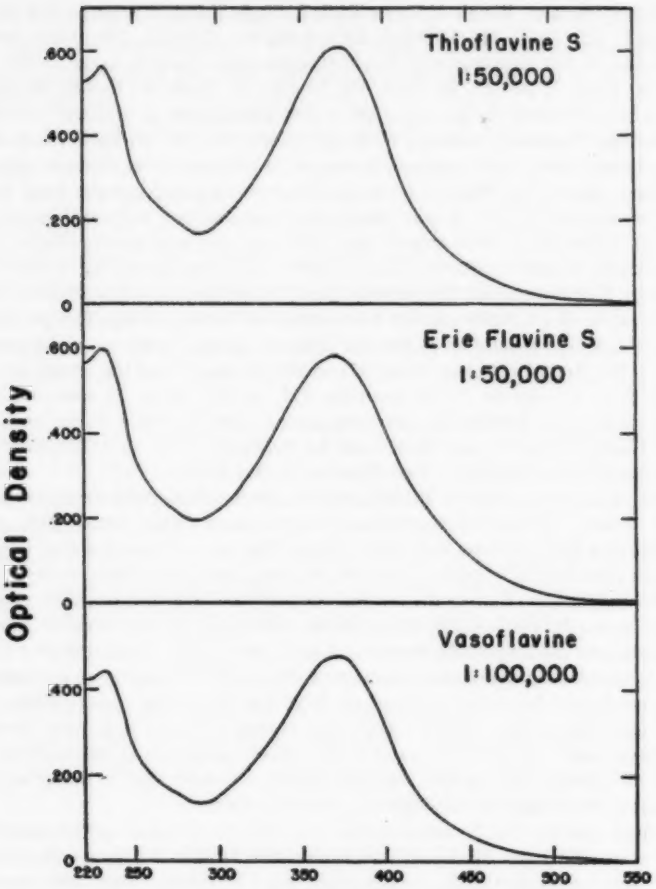


FIG. 1. Absorption spectra of thioflavine S, Erie Flavine S Techn. and vasoflavine. See Material and Methods and text.

and bluish in color, and is confined to the cytoplasm of the hepatic cells. Its character is such that it might well interfere with the detection of small amounts of thioflavine S and vasoflavine, which—when present in the cytoplasm at low intensity—exhibit a characteristic blue fluorescence. The most striking feature of the liver is the presence of more or less numerous, small, yellow or yellowish-white, fluorescent bodies (referred to as naturally fluorescent bodies) in the cells lining the hepatic sinusoids. Their intensity is variable, and initially may be quite high. However, their fluorescence is rapidly extinguished by exposure to ultraviolet light; and they do not interfere appreciably in studies of the type reported here. The possible nature of these bodies, with all necessary references to the literature, has been discussed elsewhere (3).

With our equipment, in our untreated frogs (and the same is true of mice and rats), there is never any suggestion of the bile canaliculi as either a positive or a negative image. When the canalicular system is delineated following the injection of a suitable fluorescent compound, its architecture is revealed in striking, three-dimensional pictures. The frog's laminae are provided with axial canaliculi, from which intercellular branches radiate in all directions. In earlier studies with fluorescein (15), ampulla-like enlargements of the axial canaliculi and dilatations at the distal ends of their intercellular branches were repeatedly observed, and were regarded as normal variants. In the mammal, in which the laminae are typically one hepatic cell in thickness (18–20), the fundamental architecture of the bile canalicular system is that of a continuous, never-ending, polygonal meshwork (3, 19). At the most superficial focus at which the canaliculi can be visualized, the picture is one of a more or less continuous polygonal meshwork (referred to as surface polygonal pattern) displayed in the plane of focus, and interpretable in terms of subcapsular laminae, which appear to be a general feature of the mammalian liver (3, 7, 20). In both frog and mammals there may develop, under varying experimental conditions, a wide variety of abnormal phenomena, which are in general highly repetitive in character. These have been reported in detail elsewhere (6), and brief descriptions will suffice for those which concern us here.

"Beading": more or less irregularly discontinuous rather than continuous delineation of the canaliculi. *"Tendril formation":* new development of short, delicate, blindly ending branches from the canaliculi. *"Sprouting":* literally a sprouting of numerous, small vacuoles from the canaliculi, with which many of the vacuoles remain connected for a time by delicate, dye-laden channels. *Intracellular vacuole formation:* appearance within the cytoplasm of the hepatic cells of spherical vacuoles, varying in number, size and fluorescent intensity, and arising either as a sequel to "sprouting" or at times, so far as one can tell, independently thereof. *Inhibition cells:* hepatic cells which exhibit, in comparison with the hepatic cells in general, a significantly higher level of cytoplasmic fluorescence attributable to the dye.

1. The excretion of thioflavine S and vasoflavine by the frog's liver

Absorption of the dyes from an aqueous environment, and their appearance in the liver. As previously reported (15), frogs can absorb fluorescein from an aqueous environment in sufficient quantities to confuse experiments concerned with time relationships in the excretion of the dye by the liver. In simple trials it was demonstrated that the same is true of thioflavine S and vasoflavine. Whether the absorption takes place through the skin or by mouth or both is not clear. Frogs were exposed for 22 hours, in shallow water at c. 22° C., to each of the dyes in a concentration of 1:50,000. With thioflavine S, all axial canaliculi and c. ⅓ of their intercellular branches were delineated (minority at low, majority at very low intensity). With vasoflavine, all axial canaliculi and from ⅓ to ½ of their intercellular branches were delineated, on the whole at low intensity. Neither dye was present at a detectable level in the cytoplasm of the hepatic cells, the blood plasma, or elsewhere in the liver.

Sequence of events during hepatic excretion of the dyes. Following a number of preliminary trials with both dyes, an extensive initial series of frogs was studied under uniform conditions, in order to determine the general sequence of events during the hepatic excretion of thioflavine S and vasoflavine, as well as possible

TABLE I

Observations upon frogs maintained at c. 22° C., and pithed from 1 to 96 hours after injection of 30 mg. per kg. of thioflavine S and vasoflavine

Abbreviations: IB—intercellular branches (proportion delineated); thio—thioflavine S; vaso—vasoflavine; GM—great majority; M—majority; m—minority; sm—small minority; mod—moderate; occas—occasional(ly); ND—not detectable; LUL—left upper lobe; LLL—left lower lobe.

FROG NO.	INTERVAL	DYE	DELINEATION OF BILE CANALICULI[a]		DETECTABILITY OF DYE			
			Intensity	IB	In Cytoplasm of Hepatic Cells[b]	In Plasma of Active Vessels	At Sinusoid-Hepatic Cell Interface[c]	In Kupffer Cells
	hr.							
B59	1	thio	high	GM	very low–low[d]	very low	very low	occas
B65[e]		vaso	½ mod, ½ low	½	very low	very low	very low–low	ND
B58	2	thio	high	⅔	very low–low[f, g]	very low	very low	occas
B64		vaso	GM high, sm mod	M	low[h]	very low	very low	occas
B57	3	thio	mod–high	¾	very low[i, j]	very low	occas[k]	all
B63		vaso	m high, M mod	GM	very low[i]	very low	occas[l]	all
B56	4	thio	mod	GM	very low[i]	very low[m]	occas[k]	all
B62		vaso	mod	GM	very low[n]	very low	occas[o]	all
B54	6	thio	mod–high	M	very low in sm[p]	ND	occas[q]	all
B55		vaso	mod–high	M	very low[i, r]	ND	occas[q]	all
B48	9	thio	mod	½	very low[i]	ND	occas[o]	all
B51		vaso	mod–high	⅔	very low[i]	ND	occas[o]	all
B44	12	thio	mod	M	very low	ND	ND	all
B46		vaso	mod	M	very low	ND	ND	all
B45	15	thio	mod	M	ND	ND	ND	all
B47		vaso	mod	GM	very low in sm[p]	ND	ND	all
B40	18	thio	mod	M	very low	ND	ND	all
B42[e]		vaso	mod[s]	M	very low	ND	ND	occas
B41	21	thio	mod	M	very low in sm[p]	ND	ND	all
B43		vaso	mod	½	very low in sm[p]	ND	ND	all
B38	24	thio	mod	½	very low in sm[o]	ND	occas[o]	all
B39		vaso	mod	½	very low in sm[o]	ND	occas[o]	all
B50	30	thio	low–mod	½	very low in sm[o]	ND	ND[t]	all
B53		vaso	low–mod	½	very low in sm[o]	ND	ND[t]	all
B70	39	thio	low	½	very low in sm[o]	ND	occas[o]	all
B71		vaso	low	½	very low in sm[o]	ND	occas[o]	all

TABLE I—*Continued*

FROG NO.	INTERVAL	DYE	DELINEATION OF BILE CANALICULI[a]		DETECTABILITY OF DYE			
			Intensity	IB	In Cytoplasm of Hepatic Cells[b]	In Plasma of Active Vessels	At Sinusoid-Hepatic Cell Interface[c]	In Kupffer Cells
	hr.							
B49	48	thio	M mod, m low	½	ND	ND	ND	all
B52		vaso	low–mod	½	ND	ND	ND	all
B68	60	thio	low	½	ND	ND	ND[d]	all
B69		vaso	low	½	ND[d]	ND	occas[e]	all
B60	72	thio	low	½	ND	ND	ND[d]	all
B66		vaso	low	½	ND	ND	ND[d]	all
B61	96	thio	¼–½, very low–low[u]	few	ND	ND	ND	all
B67		vaso	low	½	ND	ND	ND	all

[a] All axial canaliculi delineated unless otherwise specified.
[b] In all hepatic cells unless otherwise specified; nuclei routinely spared by the dye.
[c] Dye at interface of all laminae unless otherwise specified.
[d] 1 imbibition cell, high (LUL).
[e] Liver in general (LUL and LLL, ventral and dorsal surfaces) characterized by an ill-defined "milky" appearance, but canaliculi sharply delineated.
[f] Low only in laminae adjacent to larger vessels.
[g] 2 imbibition cells, high (LLL).
[h] 1 imbibition cell, high, in each of 2 lobes.
[i] Somewhat higher in laminae adjacent to larger vessels.
[j] 1 imbibition cell, high (LLL).
[k] Apparently unequivocal in laminae adjacent to larger vessels, suggestive at best elsewhere.
[l] Apparently unequivocal in laminae adjacent to larger vessels, also at times elsewhere.
[m] Detectable only in larger vessels.
[n] 2 imbibition cells, 1 mod, 1 high (LUL).
[o] Only in laminae adjacent to larger vessels.
[p] In laminae usually adjacent to larger vessels.
[q] Detected only after initial observations completed.
[r] 3 imbibition cells, mod, 2 in LUL, 1 in LLL.
[s] 3 isolated instances of delicate "sprouting" (LUL).
[t] Suggestive at best in laminae adjacent to larger vessels.
[u] Majority very low, in many instances extremely delicate.

differences between the two samples of dye. Pairs of frogs, receiving one or the other in the same dosage, were pithed and observed at intervals varying from 1 to 96 hours following injection; and the findings are briefly summarized in Table I.

The clarity, sharpness and beauty of the three-dimensional pictures of bile canalicular delineation by the dyes left little to be desired. The contents of the canaliculi appeared at all times to be homogeneous, and movement of the pro-

visional bile along the canaliculi was never observed. Intercellular branches were routinely delineated at essentially the same fluorescent intensity as their parent axial canaliculi. The color of the canalicular contents varied with the intensity of the fluorescence: blue at low, whitish-blue at moderate, and bluish-white at high intensity. With respect to the proportions of the intercellular branches delineated in the various animals, the fractional estimates recorded in the table are admittedly rough approximations at best. Rare or occasional ampulla-like enlargements of the axial canaliculi were encountered in a number of the animals, and appeared in all cases to be entirely equivalent—in range of size and shape, and in character—to those seen in fluorescein preparations. Dilatations at the distal ends of the intercellular branches were not noted, but could easily have been missed. The only abnormalities of the canaliculi which were observed were three isolated instances of delicate "sprouting" in one lobe of a single frog.

Deferring, for the moment, consideration of the at times distinctive properties of the sinusoid-hepatic cell interface, the cytoplasm of the hepatic cells—when it exhibited the characteristic fluorescence of the dyes—appeared quite homogeneous. Known intracellular inclusions (mitochondria, etc.) were not revealed as either positive or negative images; and there was nothing to indicate concentration of the dyes in particular regions of the cell. The nuclei of the hepatic cells were invariably displayed as dark, relatively or absolutely non-fluorescent, spherical bodies, devoid of internal structure. The more intense the fluorescence of the cytoplasm, the more clearly were the nuclei displayed by contrast. In comparison with the laminae as a whole, there was a general tendency for the laminae adjacent to larger vessels to exhibit appreciably higher cytoplasmic fluorescence attributable to the dyes. In these same laminae, the intercellular branches of the canaliculi were usually delineated rather completely when their delineation simultaneously elsewhere was variably incomplete. The only abnormalities of the hepatic cells which were encountered were rare imbibition cells in 6 of the 34 animals; their nuclei were in all instances spared by the dye. Even in the brief time (2 minutes per lobe) allotted for critical observations, a reduction in the initial intensity of canalicular delineation was frequently apparent; and, concomitantly, it was sometimes noted that the fluorescent intensity of the cytoplasm of the hepatic cells increased appreciably, and that the picture of nuclear sparing by the dyes became more bold. Furthermore, in one of the animals (B45), in which the hepatic cells exhibited initially only natural fluorescence, subsequent study revealed the gradual appearance of the dye in the cytoplasm.

When detectable in the plasma of active vessels, the dyes usually exhibited an ill-defined yellowish-white fluorescence, never exceeding very low intensity. In some of the animals there was noted an apparent concentration of the dye at the sinusoid-hepatic cell interface, in the form of a very delicate line of essen-

tially uniform thickness, whitish-yellow in color and never exceeding low fluorescent intensity. When present, this phenomenon was unmistakable.

A striking feature of the liver, in practically all of the animals studied 3 hours and more after injection, was the more or less generalized presence of numerous, small, fluorescent inclusions—varying shades of yellow-brown in color, and never exceeding moderate in intensity—in the cells lining the sinusoids, but not the larger vessels. When well developed, the picture afforded a seemingly complete display of all of the Kupffer cells; and could only be interpreted in terms of segregation and storage of the dyes within their cytoplasm. The lining cells delineated in this way were extremely numerous, and of varying thickness and shape. In many of the larger ones, the nuclear regions could be readily visualized as dark, relatively non-fluorescent areas. When selected fields were held under more or less prolonged observation, the fluorescent inclusions did not exhibit any appreciable reduction in intensity or change in color. The inclusions were far more numerous than the naturally fluorescent bodies as usually observed. In this series, the presence of the latter (in small numbers) was noted in only a single animal. However, no particular effort was made to visualize them; and they could easily have been obscured by the fluorescence of the plasma, the Kupffer cells or both.

In terms of the above descriptions, the data as recorded in Table I are for the most part self-explanatory. Although proceeding on the whole from higher to lower levels with progressively longer intervals from the time of injection, the fluorescent intensity of the canaliculi, and the proportion of intercellular branches delineated, showed considerable variability, particularly in the earlier stages of the excretory process. Some of this variability is undoubtedly attributable to the difficulty of making subjective judgments of the pictures obtained. Although indicating that the peak of excretion is reached rather early after injection, this series did not establish the peak within reasonable limits. It is noteworthy that it likewise failed to reveal any consistent or characteristic differences between the two dyes in so far as their hepatic excretion is concerned.

It seemed particularly important to obtain more detailed data upon the sequence of events in the early stages of the excretory process. To this end, a second series was accomplished under uniform conditions, with the frogs pithed at intervals from 15 minutes to 3 hours following injection, and again with the study of companion animals receiving the same dosage of the two samples of dye. The findings are summarized in Table II, and require but brief comment. Widespread delineation of the canaliculi occurs promptly, at a fluorescent intensity which develops rather rapidly to more or less of a plateau, which is well maintained for 2 hours or more. During this period, the general level of intensity is on the whole significantly higher with vasoflavine than with thioflavine S. The presence of the dyes in detectable amounts in essentially all of

TABLE II

Observations upon frogs maintained at c. 22° C., and pithed from 15 to 180 minutes after injection of 30 mg. per kg. of thioflavine S and vasoflavine

Abbreviations: as in Table I

FROG NO.	INTERVAL	DYE	DELINEATION OF BILE CANALICULI[a]		DETECTABILITY OF DYE			
			Intensity	IB	In Cytoplasm of Hepatic Cells[b]	In Plasma of Active Vessels	At Sinusoid-Hepatic Cell Interface[c]	In Kupffer Cells
	min.							
B73	15	thio	low	½	very low[d]	very low	ND	ND
B79		vaso	low	GM	very low–low[e]	very low–low	very low	ND
B74	30	thio	mod[d]	⅔	very low–low[e]	very low	very low	ND
B92		thio	low	½	very low[d]	very low	very low	ND
B80		vaso	low–mod	¾	very low–low[e]	very low	very low	ND
B85		vaso	mod	½	very low–low[e]	very low	very low	ND
B75	45	thio	high	⅔	very low–low[e]	low	very low	ND[f]
B93		thio	mod	⅔	very low[d]	very low	very low	ND
B81		vaso	mod	GM	very low–low[e]	very low–low[g]	very low	ND
B86		vaso	high	⅔	very low–low	very low–low[g]	very low–low	ND
B76	60	thio	M mod, m low	½	very low–low[e]	very low–low	very low	occas[h]
B94		thio	mod	½	very low	very low	very low	ND
B82		vaso	mod–high	GM	low[d]	very low–low[g]	very low–low	ND
B87		vaso	high	⅔	very low–low[e]	very low–low	very low–low	ND
B77	75	thio	low	⅓	very low	very low	very low	occas
B83		vaso	M high, m mod	GM	very low–low[e]	very low–low[g]	very low–low	many
B78	90	thio	mod	¾	very low	very low	very low	occas
B95		thio	mod	½	very low[d]	very low	very low[i]	all
B84		vaso	high	⅔	low	very low	very low	M
B88		vaso	high	⅔	very low–low[e]	very low	very low–low	ND[j]
B96	120	thio	mod	⅔	very low[d]	very low	very low[i]	all
B89		vaso	high	¾	very low[d]	very low	very low[k]	all
B97	150	thio	mod	⅓	very low[d]	very low	very low[i]	all
B90		vaso	high	GM	very low[d]	very low	very low[i]	all
B98	180	thio	high	½	very low	very low	very low[i]	all
B91		vaso	mod	½	very low[d]	very low	very low[i]	all

[a] All axial canaliculi delineated.
[b] In all hepatic cells; nuclei routinely spared by the dye.
[c] At interface of all laminae unless otherwise specified.
[d] Somewhat higher in laminae adjacent to larger vessels.
[e] Low only in laminae adjacent to larger vessels.
[f] In some instances suggestive at best.
[g] Low only in larger vessels.
[h] Detected only after initial observations completed.
[i] Visualized with assurance only in laminae adjacent to larger vessels.
[j] Suggestive in some instances.
[k] Apparently unequivocal in many instances.

TABLE III

Observations upon frogs maintained at c. 22° C., and pithed from 3 to 17 days after injection of 30 mg. per kg. of thioflavine S and vasoflavine[a]

Abbreviations: as in Table I

FROG NO.	INTERVAL	DYE	DELINEATION OF BILE CANALICULI[b]			DETECTABILITY OF DYE IN KUPFFER CELLS	
			Axial		IB	Low	Very low
			Low	Very low			
	days						
B99[c]	3	thio	all	—	⅓	all	—
B104[c]		vaso	all	—	½	all	—
B100	4	thio	—	all	⅓	all	—
B106		vaso	¾	¼	½	all	—
B101	5	thio	—	all	⅓	all	—
B107		vaso	¾	¼	⅓	all	—
B102	6	thio	all	—	⅓	all	—
B103		thio	—	all	⅓	all	—
B108		vaso	—	all	⅓	all	—
B110	7	thio	—	all	⅓	m	M
B116		vaso	all	—	¼	all	—
B111	9	thio	—	all	⅓	—	all
B117[d]		vaso	m	M	¼	—	all
B112	11	thio	—	all	⅓	—	all
B119		vaso	—	all	¼	—	all
B113	14	thio	—	all	¼	—	all
B120		vaso	—	all	¼	—	all
B114	17	thio	—	⅔	1/10	—	all
B121		vaso	—	½	Rare	—	all

[a] Of the 22 frogs used for this study, 3 were found dead, as follows: B105, vaso, 2nd day; B115, thio, and B118, vaso, both on 5th day.

[b] With few exceptions, intercellular branches delineated at essentially the same intensity as their parent axial canaliculi.

[c] Presence of dye suggestive in hepatic cells and apparently unequivocal at sinusoid-hepatic cell interface in laminae adjacent to larger vessels.

[d] LUL: all axials and c. ⅓ of IB delineated, m at low, M at very low intensity. LLL: c. ¾ of axials and, in same laminae, c. ½ of IB delineated at very low intensity. Regions showing no delineation of canaliculi were distributed irregularly over both ventral and dorsal surfaces of LLL.

the Kupffer cells is achieved roughly 2 hours following injection, at which time the fluorescent intensity of the inclusions is rather low compared with that observed in frogs pithed after longer intervals.

Naturally fluorescent bodies of the usual sort were noted in from very small

TABLE IV

Observations upon frogs maintained at c. 22° C., and pithed 45 minutes after injection of from 0.47 to 120 mg. per kg. of thioflavine S and vasoflavine

Abbreviations: as in Table I

FROG NO.	DOSAGE	DYE	DELINEATION OF BILE CANALICULI[a]		DETECTABILITY OF DYE		
			Intensity	IB	In Cytoplasm of Hepatic Cells[b]	In Plasma of Active Vessels	At Sinusoid-Hepatic Cell Interface[c]
	mg. per kg.						
B123[d]	0.47	thio	sm very low[e]	ND	ND[f]	ND	ND
B124[d]		vaso	ND	ND	ND	ND	ND[g]
B125	0.94	thio	very low[h]	1/10	ND	ND	ND
B126		vaso	very low	1/4	very low[i]	ND	occas[j]
B127	1.88	thio	very low	1/5	ND	ND	ND
B128		vaso	very low	1/5	ND	ND	ND
B129	3.75	thio	low	1/2	ND	ND	very low[k]
B130		vaso	low	1/2	very low[l]	ND	ND
B131	7.5	thio	low	2/3	ND[m]	very low	very low
B132		vaso	low–mod	2/3	very low in sm[n]	very low	very low
B133	15	thio	low–mod	2/3	M very low, sm low[o, p]	very low	very low[q]
B134		vaso	low	1/3	very low in sm[j]	very low	very low
B135	30	thio	GM mod, sm mod–high	2/3	GM very low, sm low[j]	very low–low	very low[q]
B136		vaso	mod	1/2	GM very low, sm low[j]	very low	very low[q]
B137	60	thio	high	2/3	GM low, sm mod[j, r]	low–mod[s]	low
B138		vaso	high	3/4	GM low, sm low–mod[j, t]	very low	very low
B139	120	thio	high	All	GM low, sm mod[j, u]	very low–low[v]	low
B140		vaso	high	All	GM mod, sm high[j, w]	low–mod[s]	high

[a] All axial canaliculi delineated unless otherwise specified.

[b] In all cells unless otherwise specified; nuclei routinely spared by dye.

[c] At interface of all laminae unless otherwise specified.

[d] Ventral and dorsal surfaces of LUL and LLL surveyed in turn, with essentially the same findings in each lobe (see footnote e).

[e] In LUL, no more than 1/10 of axial canaliculi delineated, most of them barely detectable, a few

to modest numbers in 7 of the 26 animals, including both thioflavine S and vasoflavine preparations, and all of them within the first 45 minutes with one exception (B77, 75 minutes). As in the first series, rare or occasional ampulla-like enlargements of the axial canaliculi were encountered in a number of the animals (17 out of 26); and again, no instance was noted of dilatations at the distal ends of intercellular branches. No abnormalities of the canaliculi were detected; and the only abnormalities of the hepatic cells which were observed were imbibition cells, in 14 of the 26 frogs (from 1 to 7 per lobe and not exceeding 7 per animal, with one exception: B98, in which 20 such cells were present within a single microscopic field).

As recorded in Table I, both thioflavine S and vasoflavine were still present in significant amounts, in both the bile canaliculi and the Kupffer cells, 4 days after the injection of 30 mg. per kg. In order to explore more fully the later stages of the excretory process, a third series of frogs was studied, again under uniform conditions and with companion animals receiving the same dosage of the two samples of dye. The frogs were pithed at intervals from 3 to 17 days following injection, and the findings are summarized in Table III. It is at once

---

(in laminae adjacent to larger vessels) at up to very low intensity. In LLL, only rare axials delineated, barely detectable.

[f] In LUL, ventral surface, 1 instance of intracellular vacuole formation (1 vacuole, medium size, low intensity).

[g] On both ventral and dorsal surfaces of LUL and LLL, a curious, barely detectable picture of ill-defined, yellowish fluorescence along the margins of the sinusoids, suggesting presence of the dye in the Kupffer cells.

[h] Canaliculi delineated on the whole at somewhat lower intensity in LLL than in LUL.

[i] In most of the hepatic cells the cytoplasm exhibits an ill-defined yellowish fluorescence of very low intensity. In laminae adjacent to larger vessels, the cytoplasm exhibits the usual bluish fluorescence of the dye.

[j] Only in laminae adjacent to larger vessels.

[k] Apparently unequivocal not only in laminae adjacent to larger vessels, but also in c. ½ of remaining laminae.

[l] Routinely exhibits ill-defined, yellowish fluorescence of very low intensity (see B126, footnote i).

[m] 1 imbibition cell, bet. low and mod intensity (LLL).

[n] In 1 small lamina the hepatic cells contain from 1 to several inclusions, of small or medium size, exhibiting yellow fluorescence of moderate intensity. Located in region between nucleus and sinusoidal wall. Associated canaliculi well delineated, normal in appearance.

[o] Not detectable with assurance in c. ¼ of hepatic cells.

[p] 1 imbibition cell, mod (LUL).

[q] In occasional instances, suggestive yellowish fluorescence of Kupffer cells.

[r] In 1 field 2 imbibition cells, high; in another 7, 3 mod, 4 high (LUL).

[s] Low in sinusoids, up to mod in larger vessels.

[t] In 1 field 5 imbibition cells, 1 mod, 4 high (LUL).

[u] In 1 field 3 imbibition cells, 1 mod, 2 high, and a lamina exhibiting moderately coarse "sprouting" (LUL).

[v] In a sinusoid an amorphous body of medium size, and exhibiting orange fluorescence of bet. mod and high intensity (particle of dye?).

[w] Canaliculi poorly displayed for lack of contrast with cytoplasm.

apparent that the dyes persist for a long time in the canaliculi and in the Kupffer cells, a portion of the former and all of the latter being still delineated on the 17th day. It is possible, of course, that the Kupffer cells themselves slowly release their dye content in sufficient amounts to achieve continuing delineation of the canaliculi over a protracted period. However, a striking feature of all of the animals was the fact that the gall bladder, which was always well filled, contained large amounts of the dye, usually at high fluorescent intensity and yellowish-white in color. Slow and continuous reabsorption of the dyes from such a depot would appear to afford an adequate explanation for the persistence of the dyes in the liver. No abnormalities of the canaliculi or of the hepatic cells were encountered, and there was nothing to suggest toxic effects from the dyes in these long-term experiments.

Studies with graded dosage of the dyes. The liver was studied in frogs which received from 0.47 to 120 mg. per kg. of either thioflavine S or vasoflavine (the dosage being doubled for each succeeding pair of animals), and which were pithed at a standard interval (45 minutes, which appeared to be a reasonable compromise from the data in Table II). The findings are summarized in Table IV, and are largely self-explanatory. In so far as the fluorescent images obtained in the liver are concerned, there would appear to be little difference between the dyes, irrespective of the level of dosage over a very wide range.

Of interest is the fact that, in two of the frogs receiving small amounts of vasoflavine, most (B126) or all (B130) of the hepatic cells exhibited an unusual yellowish fluorescence of their cytoplasm. It is also noteworthy that the boldest picture of apparent concentration of the dye at the sinusoid-hepatic cell interface was observed in the frog receiving the highest dose of vasoflavine (B140). When detectable in the plasma of active vessels, both dyes consistently exhibited an ill-defined yellowish fluorescence (yellowish-white at moderate intensity in B137). The previously reported instance of blue fluorescence of the plasma (3) remains unique in our experience. As noted earlier in similar studies with fluorescein (15), the caliber of the canaliculi was no larger at higher dosage than in a standard 30 mg. per kg. preparation; and the canaliculi exhibited no other appreciable difference in character. They were, however, delineated at significantly lower dosage of thioflavine S and vasoflavine than of fluorescein. Rare or occasional ampulla-like enlargements of the axial canaliculi were noted in frogs B127 and B129–B140 inclusive, and were entirely within the normal range.

Studies at low temperature (c. 1° C.). Frogs were maintained in shallow water at c. 1° C. for from 18 to 20 hours prior to the injection of vasoflavine in a dosage of 30 mg. per kg.; and refrigeration was continued until the animals were pithed for study at intervals from 15 minutes to 4 hours later. At this reduced temperature, the liver showed a surprising ability to transfer the dye into the provisional bile and, with time, to concentrate it in high degree. The following

protocols are self-explanatory. In a frog injected with thioflavine S under the same conditions and pithed 4 hours later, the findings differed in two respects from those described below (B141) with vasoflavine: the canaliculi were delineated on the whole at somewhat lower intensity, and concentration of the dye at the sinusoid-hepatic cell interface was suggestive at best, even in laminae adjacent to larger vessels.

B155, 15 minutes—Left upper lobe (LUL): c. ½ of axial canaliculi (AC), distributed irregularly over surface, delineated (del.) at barely detectable (det.) level. No intercellular branches (IB) del. Dye not det. in cytoplasm of hepatic cells (HC), at sinusoid-hepatic cell interface or in Kupffer cells (KC). Left lower lobe (LLL): c. ¾ of AC and c. ⅕ of IB del. at from barely det. level to very low intensity (int.). Dye det. in HC (*very* low) only in laminae (lam.) adjacent (adj.) to larger vessels (vv.).

B153, 30 minutes—LUL: All AC and c. ⅙ of IB del., majority (maj.) at very low, minority (min.) at low int. Dye det. in HC (*very* low) only in lam. adj. to larger vv.; not det. at interface or in KC. LLL: c. ⅗ of AC and c. $\frac{1}{10}$ of IB del., maj. at very low, min. at low int. Dye det. in HC (*very* low) only in lam. adj. to larger vv.; not det. at interface or in KC.

B154, 1 hour—LUL and LLL: All AC and c. ⅙ of IB del., maj. at low, min. at moderate (mod.) int. Dye det. in all HC, maj. at very low, min. (in lam. adj. to larger vv.) at low int.; det. (very low) in plasma of active vv.; not det. at interface or in KC.

B156, 2 hours—LUL and LLL: All AC and c. ⅗ of IB del., mod. int. Dye det. in all HC, maj. at very low, min. (in lam. adj. to larger vv.) at low int.; det. (*very* low) in plasma of active vv.; not det. at interface or in KC.

B141, 4 hours—LUL and LLL: All AC and great maj. of IB del. at high int. Dye det. in all HC, maj. at low, min. (in lam. adj. to larger vv.) at mod. int.; det. in plasma of active vv. (very low in sinusoids, approaching low int. in larger vv., yellowish in color) and with few exceptions at interface (delicate, very low int., yellowish). Dye not det. with assurance in KC (occasionally suggestive).

2. The excretion of vasoflavine by the liver of the white mouse

The normal sequence of events in the liver of the white mouse, following the intraperitoneal injection of thioflavine S 1:1,000 in a dosage of 30 mg. per kg., has been previously reported (3, 4). In contrast to fluorescein and esculin, which (in the same dosage) are rather rapidly cleared from the liver (fluorescein in 1½ hours or less, esculin in an hour or less), thioflavine S persists in sufficient amounts to delineate at least some of the bile canaliculi for 24 hours or more. For comparison with these earlier data, and utilizing the same technical procedures throughout, observations have been made upon the liver of mice sacrificed at intervals following the intraperitoneal injection of vasoflavine. The findings are summarized in Table V, and are in surprisingly close agreement with those for thioflavine S. In none of the animals was there any suggestion of either concentration of the dye at the sinusoid-hepatic cell interface, or segregation of the dye in the Kupffer cells.

In a small series of mice the liver was studied *in vivo*, under chloral hydrate anesthesia (0.45 or 0.60 gm. per kg., administered subcutaneously), following the intravenous injection

TABLE V

Observations upon white mice sacrificed from 5 minutes to 30 hours after intraperitoneal injection of 30 mg. per kg. of vasoflavine

MOUSE NO.	INTERVAL	INTENSITY OF VASOFLAVINE						
		In Bile Canaliculi				In Cytoplasm of Hepatic Cells		
		High	Moderate	Low	Very low	Moderate	Low	Very low
	min.							
B5[a]	5	—	½	½	Few	¼	¾	—
B6[a]	10	¾	¼	—	—	¼	¾	—
B7[b]	15	⅜	⅛	—	—	⅛	⅜	—
B8[a, c]	20	¾	¼	—	—	⅛	⅞	—
B9[a, c]	25	¾	¼	—	—	⅛	⅞	—
B10[a]	30	¾	¼	—	—	—	All	—
B11[a, c]	40	—	All	—	—	—	All	—
B12[a, c, d]	50	—	¾	¼	—	—	All	—
B13[a]	60	—	All	—	—	—	All	—
B14[a, c]	90	—	⅜	⅛	—	—	⅜	⅛
	hr.							
B15	2	—	¾	¼	—	—	½	½
B16[a, c]	3	—	½	⅛	—	—	⅛	⅜
B17[a, c, e]	4	—	—	½	½	—	—	⅛
B18[a, c, f]	6	—	—	⅛	⅞	—	—	—
B19	9	—	—	—	All	—	—	—
B20[g]	12	—	—	—	All	—	—	—
B21[g]	15	—	—	—	All	—	—	—
B22[a, c, g]	18	—	—	⅙	⅚	—	—	—
B23[h]	21	—	—	—	¾	—	—	—
B24[h, i]	24	—	—	—	1/10	—	—	—
B25[h]	27	—	—	—	½	—	—	—
B26[h]	30	—	—	—	—	—	—	—
B27[h]	30	—	—	—	—	—	—	—

[a] Occasional instances of delicate "beading" noted in surface polygonal pattern.

[b] 3 isolated imbibition cells (moderate intensity, nuclei spared by dye), 2 instances of delicate "sprouting", and 1 instance of intracellular vacuole formation (1 vacuole, medium size, moderate intensity) noted in surface polygonal pattern.

[c] Rare instances of "tendril formation" in surface polygonal pattern.

[d] 1 instance of intracellular vacuole formation (2 vacuoles, medium size, high intensity) in surface polygonal pattern.

[e] Dye detectable in cytoplasm of hepatic cells only in small groups of laminae distributed regionally over surface.

[f] Presence of dye in cytoplasm of hepatic cells suggestive at best in small groups of laminae distributed regionally over surface.

[g] Left lower and left upper lobes surveyed in turn, with essentially the same findings.

[h] Left lower, left upper and right upper lobes surveyed in turn, with essentially the same findings.

[i] In right upper lobe, an isolated group of 4 imbibition cells (low intensity, nuclei spared by dye) noted in surface polygonal pattern; associated canaliculi delineated at low intensity.

(tail vein) of vasoflavine 1:500 in a dosage of 30 mg. per kg. Exposure of the liver was begun from 5 to 10 minutes after injection of the dye had been completed. Necessary procedures for securing hemostasis and for stabilizing the organ are time-consuming, and the liver was first brought under observation from 4 to 8 minutes after the beginning of operation. In each

case, after the *in vivo* studies had been completed, the animal was sacrificed (by opening the pleural cavities) and the same lobes of the liver were promptly re-examined at sharp focus, free from interference by transmitted movements. The most important information derived from these studies was that the caliber and character of the canaliculi were essentially the same under anesthesia, with good intrahepatic circulation, as in unanesthetized mice observed soon after sacrifice. Also, the fluorescent images of the hepatic cells were entirely comparable under the two sets of conditions. There was no suggestion, *in vivo*, of dye concentration at the sinusoid-hepatic cell interface or of its segregation in the Kupffer cells.

3. Absorption spectra of thioflavine S and vasoflavine

For the more exact characterization of the dyes used in this study, absorption spectra of the samples of thioflavine S and vasoflavine were determined spectrophotometrically by standard methods. The spectra are illustrated in Figure 1, which includes for comparison that of a sample of Erie Flavine S Techn. (their trade name for thioflavine S) obtained from National Aniline. In its spectral characteristics, our vasoflavine appears to be essentially equivalent to that used in their original study by Moses, Emery and Schlegel (13).

4. Studies with thioflavine T and primuline

Exploratory studies of the liver were made in frogs injected with thioflavine T and primuline, in dosages of 60 and 120 mg. per kg., and pithed 30 minutes later. Even at the higher dosage, thioflavine T—which exhibits in aqueous solution (1:500) a soft greenish-yellow fluorescence—did not appear in the liver in detectable amounts. Primuline, on the other hand, yielded pictures of the canaliculi, hepatic cells and sinusoid-hepatic cell interface which were similar in most respects to those obtained with thioflavine S and vasoflavine. However, since essentially equivalent pictures could be regularly achieved with the latter dyes at far lower dosage, and since primuline did not appear to make possible the visualization of any novel features of hepatic structure or of the excretory process, studies with this dye were discontinued. Detailed observations were as follows in a frog receiving primuline in a dosage of 120 mg. per kg.:

Left upper lobe, ventral surface: All canaliculi delineated at moderate fluorescent intensity and bluish-white in color, with a yellowish cast; entirely normal in caliber and otherwise. All hepatic cells exhibit the dye in their cytoplasm, usually at low intensity (sometimes higher) and bluish-white in color; the nuclei are routinely spared. There is an apparent concentration of the dye at the sinusoid-hepatic cell interface, appearing as a thin line of from low to moderate intensity, and greenish-yellow in color. The dye is detectable in the plasma of active vessels, at very low or low intensity and bluish-white in color. No abnormalities encountered in wide survey (3 minutes). *Left lower lobe, ventral surface:* Findings essentially the same as above except that the level of fluorescent intensity of the canaliculi is on the whole somewhat lower.

DISCUSSION

In these attempts to establish the normal sequence of events during the hepatic excretion of thioflavine S and vasoflavine, we have relied most entirely

upon rapid evaluation of the initial fluorescent images in frogs pithed at intervals following injection of the dyes, and brought under observation as quickly as possible. The basis for this procedure derives principally from earlier studies (3, 6, 15), in which it was demonstrated that a wide variety of abnormal phenomena may develop when the liver is subjected to more or less prolonged observation under the fluorescence microscope. In addition, the particular dyes with which we are here concerned are quite photosensitive; the initially sharp pictures of bile canalicular delineation are rather rapidly suppressed upon exposure to ultraviolet light, and may be totally extinguished within a few minutes or less. Concomitantly, one frequently observes an appreciable increase in the fluorescent intensity of the dyes in the cytoplasm of the hepatic cells.

Under the circumstances, it is quite impossible to make detailed studies of selected microscopic fields, during the excretory process, with any assurance that one is observing the normal sequence of events. On the contrary, one has every reason to believe that he is certainly entering the realm of abnormality within a matter of seconds after bringing any particular field into sharp focus. In our experience, in any attempt to make continuous observations in a given animal, the best device is to keep moving steadily over the surface of the liver, always into hitherto unexposed areas. Considerable information of interest concerning the development of the fluorescent images can be obtained in frogs studied in this way following injection of the dyes (3). However, one is always suspicious of the validity of such observations, and further studies along these lines were discontinued. An alternative device is to expose a selected microscopic field very briefly and repeatedly, at appropriate intervals. Even here, if the field is observed on each occasion for a sufficient length of time to permit reasonably accurate evaluation, significant changes attributable to the exposure soon become apparent.

In so far as the fluorescent images obtained in the frog's liver are concerned, thioflavine S and vasoflavine behave in an essentially equivalent manner. With the same dosage of the two samples of dye, the sequence of events (including time relationships) is so entirely similar that it makes no appreciable difference which dye is employed in studies of hepatic excretion by fluorescence microscopy. The same is true when the two dyes are tested over a very wide range of dosage. As mentioned in the introduction, Moses *et al.* (13, 14) have found vasoflavine to give far more striking pictures of blood vessel walls than thioflavine S. However, they utilize very high concentrations of the dyes, and are concerned primarily with the pictures obtained immediately after the injection has been completed. These conditions are not applicable to studies of hepatic excretion of the type reported here.

From the considerations of Moses *et al.* (13) concerning the state of thioflavine S and vasoflavine in aqueous solutions, our observations would indicate

that it is principally if not exclusively the monomeric form of the dyes which passes the sinusoidal barrier, and is transferred by the hepatic cells into the bile canaliculi. As the concentration of the dyes increases in the canaliculi, the fluorescence shifts from blue at low intensity to a bluish-white at high intensity. In the pictures of apparent segregation of the dyes in the cytoplasm of the Kupffer cells, the inclusions exhibit fluorescence which is varying shades of yellow-brown in color. This unusual fluorescence is presumably attributable to a particular physical state of the dyes. (It should be mentioned that in the solid state both dyes are characterized by an ill-defined orange fluorescence.) Being principally concerned with the fluorescent images of the hepatic cells and bile canaliculi and with time relationships during the excretory process, we have made no special study of the Kupffer cell pictures other than to record their presence, extent and fluorescent intensity. However, when well developed the pictures are quite striking indeed, and obviously merit further investigation.

Although differing in time relationships and in certain other features, the general sequence of events during the hepatic excretion of fluorescein on the one hand (15), and of thioflavine S and vasoflavine on the other, is fundamentally similar under normal conditions. Particularly is this true of the delineation of the bile canaliculi and of the appearance of the dyes in the cytoplasm of the hepatic cells. However, recent studies in mice have demonstrated a noteworthy difference between the dyes in extrahepatic biliary obstruction (5). Even early in obstruction, it proved virtually impossible to achieve delineation of the bile canaliculi with fluorescein, and the dye frequently failed to appear in the hepatic cells at a detectable level. With vasoflavine, on the other hand, the bile canaliculi could usually be delineated quite well at any stage of biliary obstruction.†

SUMMARY

Utilizing the technic of fluorescence microscopy, the hepatic excretion of thioflavine S—in both its conventional and its more purified form (vasoflavine)—has been investigated by *in vivo* observations upon the frog's liver at varying intervals following injection of the dyes. The general sequence of events is similar to that observed with fluorescein under comparable conditions of dosage, temperature and experimental procedure. However, thioflavine S and vasoflavine are cleared from the liver much more slowly, and in addition are apparently segregated in considerable amounts in the cytoplasm of the Kupffer cells. In so far as the fluorescent images obtained in the frog's liver are con-

† We are indebted to Dr. Edward Singer for calling to our attention his recent report (21) upon the use of fluorescence microscopy in the investigation of electrical phenomena in cells and tissues. The report includes a general consideration of the factors involved in vital staining with a variety of fluorescent compounds, including thioflavine S, fluorescein and esculin; and supplements an earlier report by Keller and Singer (22) along similar lines.

cerned, thioflavine S and vasoflavine—at the same dosage, over a very wide range—behave in an essentially equivalent manner. The observations indicate that it is principally if not exclusively the monomeric form of the dyes which passes the sinusoidal barrier, and is transferred by the hepatic cells into the bile canaliculi.

LITERATURE CITED

1. Ellinger, P. and Hirt, A.: Mikroskopische Untersuchungen an lebenden Organen. I. Mitteilung. Methodik: Intravitalmikroskopie. Zeitschr. f. Anat. u. Entw., 1929, **90:** 791–802.
2. Ellinger, P. and Hirt, A.: Eine Methode zur Beobachtung lebender Organe mit stärksten Vergrösserungen im Luminiszenzlicht (Intravitalmikroskopie). Abderhalden's Handb. d. biol. Arbeitsmeth., 1930, Abt. V, Teil 2/2: 1753–1764.
3. Grafflin, A. L. and Bagley, E. H.: Studies of hepatic structure and function by fluorescence microscopy. Bull. Johns Hopkins Hosp., 1952, **90:** 395–437.
4. Bagley, E. H. and Grafflin, A. L.: The mechanism of biliary excretion in mammals. *Liver Injury. Transactions of the Eleventh Conference.* Hoffbauer, F. W., ed. Josiah Macy, Jr. Foundation, New York, 1953: 200–215.
5. Grafflin, A. L. and Chaney, V. E., Jr., with the assistance of Corddry, E. G.: Studies of extrahepatic biliary obstruction in the white mouse by fluorescence microscopy. Bull. Johns Hopkins Hosp., 1953, **93:** 107–139.
6. Grafflin, A. L. and Corddry, E. G.: Further studies of hepatic structure and function by fluorescence microscopy. Bull. Johns Hopkins Hosp., 1953, **93:** 205–224.
7. Hanzon, V.: Liver cell secretion under normal and pathologic conditions studied by fluorescence microscopy on living rats. Acta Physiol. Scand., 1952, **28:** Suppl. 101: 1–268.
8. Singer, E.: Histological aspects of the function of the malpighian body in the living frog's kidney, based on studies with the fluorescence microscope. Anat. Rec., 1936, **66:** 343–368.
9. Singer, E.: Photodynamic effects of fluorescent substances in the living kidney. Arch. Path., 1936, **22:** 813–816.
10. Schlegel, J. U.: Demonstration of blood vessels and lymphatics with a fluorescent dye in ultraviolet light. Anat. Rec., 1949, **105:** 433–443.
11. Algire, G. H. and Schlegel, J. U.: Circulatory reactions in photodynamic action. J. Cell. and Comp. Physiol., 1950, **35:** 95–110.
12. Schlegel, J. U. and Moses, J. B.: A method for visualization of kidney blood vessels applied to studies of the crush syndrome. Proc. Soc. Exp. Biol. & Med., 1950, **74:** 832–837.
13. Moses, J. B., Emery, A. J. and Schlegel, J. U.: A purified fraction of thioflavine-S (vasoflavine): valuable agent for visualization of blood vessels. Proc. Soc. Exp. Biol. & Med., 1951, **77:** 233–239.
14. Moses, J. B. and Schlegel, J. U.: Preservation of the juxtamedullary circulation following ligation of the renal artery in the rabbit. Anat. Rec., 1952, **114:** 149–156.
15. Grafflin, A. L.: The excretion of fluorescein by the liver under normal and abnormal conditions, observed in vivo with the fluorescence microscope. Am. J. Anat., 1947, **81:** 63–116.
16. Holtfreter, J.: Über die Aufzucht isolierter Teile des Amphibienkeimes. II. Züchtung von Keimen und Keimteilen in Salzlösung. Arch. f. Entwicklungsmech., 1931, **124:** 404–466.

17. ROWE, F. M., ed.: *Colour Index*. Society of Dyers and Colourists, Bradford, England (Yorkshire), 1924.
18. ELIAS, H.: A re-examination of the structure of the mammalian liver. I. Parenchymal architecture. Am. J. Anat., 1949, **84:** 311–333.
19. ELIAS, H.: A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary systems. Am. J. Anat., 1949, **85:** 379–456.
20. ELIAS, H.: Morphology of the liver. *Liver Injury. Transactions of the Eleventh Conference.* Hoffbauer, F. W., ed. Josiah Macy, Jr. Foundation, New York, 1953: 111–199.
21. SINGER, E.: Electrocytology as revealed by fluorescence microscopy. Medical Times, 1951, **79:** 32–40.
22. KELLER, R. AND SINGER, E.: The rôle of the electrical potential of cells and tissue fluids in normal and pathologic metabolism. A guide to rational preoperative and postoperative therapy. Am. J. Surg., 1939, **43:** 170–198.

IN VIVO STUDIES OF THE FROG'S LIVER DURING THE EXCRETION OF ESCULIN*

ALLAN L. GRAFFLIN AND ELLEN G. CORDDRY

Department of Anatomy, The Johns Hopkins University School of Medicine

Received for publication October 26, 1953

As a supplement to earlier studies upon the hepatic excretion of fluorescent compounds in amphibia and mammals, the present report is concerned with the sequence of events during the excretion of the glycoside, esculin, as observed *in vivo* in the frog's liver by the technic of fluorescence microscopy. Although some detailed information is available for mammals, the data for amphibia are quite inadequate, and are limited to the early stages of the excretory process (1). All necessary comments and references to the literature for general orientation in the field, as well as details upon equipment and methods of procedure, are included in the accompanying article upon the excretion of thioflavine S and vasoflavine (2), and need not be repeated here. Male specimens of *Rana pipiens* were used throughout. All observations were made with the 55× water immersion objective and the 6× monocular. Esculin (highest purity, hydrate, lot no. 2305) was obtained from the Pfanstiehl Chemical Co. Solutions were always freshly prepared before use, and were routinely administered into the dorsal lymph sac, in a concentration of 1:1,000 except in frogs B207-B218 (Table II). For these animals a 1:1,000 solution was serially diluted, each time to half-strength, to concentrations of from 1:2,000 (for 15 mg. per kg.) to 1:64,000 (for 0.47 mg. per kg.), to facilitate absorption of the compound from the dorsal lymph sac under comparable conditions (see 2).

Esculin imparts to the plasma a fluorescence which is blue at low and whitish-blue at high intensity. When present in considerable amounts, in a liver with good circulatory activity, it so dominates the picture that it is often extremely difficult to evaluate the intensity level of the bile canaliculi and the proportion of the intercellular branches delineated. Against the luminous background of the plasma, as noted elsewhere (1), the hepatic laminae frequently appear essentially devoid of fluorescence or may exhibit an ill-defined brownish cast. We have yet to detect, with any assurance, the presence of esculin's characteristic fluorescence in the cytoplasm of the hepatic cells. That esculin may afford striking demonstrations of the intrahepatic circulation, there is no doubt; but these do not concern us here. In the bile canaliculi its color likewise varies with

* The "Ultropak" microscope and accessories employed in this study were made available on loan from the Armed Forces Institute of Pathology. We are indebted to Miss Virginia V. Croker for patient secretarial assistance with the protocols.

TABLE I

Observations upon frogs maintained at c. 23° C., and pithed from 5 minutes to 27 hours after injection of 30 mg. per kg. of esculin[a]

Abbreviations: IB—intercellular branches (proportion delineated); GM—great majority; M—majority; m—minority; sm—small minority; mod—moderate; occas—occasional; ND—not detectable; LUL—left upper lobe; LLL—left lower lobe.

FROG NO.	INTERVAL	DELINEATION OF BILE CANALICULI[b]		DETECTABILITY OF ESCULIN IN PLASMA OF ACTIVE VESSELS
		Intensity	IB	
	min.			
B166	5	ND[c]	ND	very low–low
B190	5	very low[d]	occas[d]	low–mod
B191	5	ND[e]	ND[e]	low–mod[f]
B167	10	low–mod	⅗	low–mod
B206	10	½ very low	rare	mod
B168	15	mod	⅗	mod
B192	20	mod	⅗	low–high[g]
B193	25	mod	⅗	mod–high
B169	30	mod–high	⅗	low–mod
B204	30	low–mod	½	mod–high
B205	35	mod	⅓	mod
B194[h]	40	mod–high	½	mod
B170	45	mod–high	⅗	low–mod
B195	50	mod	⅓	very low–low
B171	60	low–mod[i]	½	mod–high
B196	60	GM mod, sm low–mod	¼	low–mod
B197[j]	60	low–mod	¼	very low–low
B172	75	mod[k]	½	low–mod
B173	90	mod[k]	½	low–mod
B174	120	mod	½	low–mod
B175[l]	150	mod[k]	⅓	low
	hr.			
B176	3	low[m]	⅕	low
B177	4	low	⅒	low
B178[n]	6	low	⅒	very low
B179[o]	9	very low	occas	very low
B201[p]	9	very low	occas	ND
B202	9	very low	occas	ND
B203	9	very low	occas	ND
B180	12	very low	ND	ND
B181	15	very low	occas	ND
B182	18	occas, very low–low[q]	ND	ND
B198	18	ND	ND	ND
B183	21	rare, very low[r]	ND	ND
B199	21	ND	ND	ND
B184	24	rare, very low[s]	ND	ND
B200	24	ND	ND	ND
B185	27	ND	ND	ND

[a] Weight range 17.2–32.0, average 23.5 gm. Delay from initiation of pithing to first visualization of liver (LUL): range 70–110, average 81 seconds. Delay from completion of observations upon

Footnotes continued on following page

the fluorescent intensity, being blue at very low and low, whitish-blue at moderate, and bluish-white at high intensity. The pictures of canalicular delineation are sharp, and are entirely equivalent to those obtained with fluorescein, thioflavine S and vasoflavine. With the latter two dyes one repeatedly encounters apparent concentration at the sinusoid-hepatic cell interface and segregation in the Kupffer cells. Neither of these phenomena has been observed with esculin.

The general sequence of events in the liver, as derived from frogs pithed at intervals following the injection of esculin in dosage of 30 mg. per kg., is illustrated by the data summarized in Table I. These are largely self-explanatory, and require but brief comment. With respect to the bile canaliculi, in the early stages of the excretory process, the findings are in some instances rather erratic; and this is presumably attributable, at least in part, to the difficulty of making the subjective judgments required (see above). The fractional estimates of intercellular branches delineated are rough approximations at best. In the presence of a rather high plasma level, we regard them as considerably less reliable than those with fluorescein, thioflavine S and vasoflavine. Many of the frogs exhibit rare, occasional or even more numerous ampulla-like en-

---

LUL to first visualization of LLL: range 5–60, average 25 seconds. Studies accomplished in July.

[b] All axial canaliculi delineated unless otherwise specified.

[c] Canaliculi not delineated during initial observations upon LUL (completed 2 minutes 40 seconds after pithing). In LLL, all axial canaliculi (but no IB) delineated at very low intensity when observations begun 3 minutes 10 seconds after pithing.

[d] Status at very beginning of observations upon LUL. Picture developed with time, and c. ¼ of IB delineated by end of 2 minutes.

[e] Canaliculi still not delineated upon completion of observations upon LLL (4 minutes 45 seconds after pithing).

[f] Low at very beginning of observations upon LUL, increasing to mod intensity by end of 2 minutes.

[g] Low to mod in LUL, mod to high intensity in LLL.

[h] 3 instances of intracellular vacuole formation, 1 in LUL, 2 in LLL.

[i] Intensity of canaliculi overshadowed by that of plasma.

[j] In LUL, 5 scattered imbibition cells, mod intensity, nuclei not visualized.

[k] A few each at high and low intensity.

[l] 1 instance of intracellular vacuole formation, LUL.

[m] A few at mod intensity.

[n] 2 isolated laminae in 1 field showing intracellular vacuole formation and apparently unequivocal "sprouting", LUL.

[o] On both ventral and dorsal surfaces of LUL and LLL, widespread, striking display of cells lining sinusoids by granular yellow fluorescence of their cytoplasm, of low to mod intensity and not extinguishing upon exposure to ultraviolet light. It appears obvious from companion animals and from the series as a whole that this unusual picture is in no way attributable to esculin.

[p] 3 instances of intracellular vacuole formation, LUL.

[q] On ventral surface of LUL, occas mid-axial accumulations of esculin, usually small, rarely of medium size, very low to low intensity; from size and shape, appear to be persistent ampulla-like enlargements of axial canaliculi. 1 such accumulation on dorsal surface of LUL, none on either surface of LLL.

[r] 1 accumulation of esculin (see footnote q) on ventral surface of LUL.

[s] Axial canaliculus at very low intensity in an isolated lamina of LUL.

TABLE II

Observations upon frogs maintained at c. 22° C., and pithed 45 minutes after injection of from 0.47 to 120 mg. per kg. of esculin[a]

Abbreviations: as in Table I.

FROG NO.	DOSAGE	DELINEATION OF BILE CANALICULI[b]		DETECTABILITY OF ESCULIN IN PLASMA OF ACTIVE VESSELS
		Intensity	IB	
	mg. per kg.			
B207	0.47	very low[c]	¼	very low
B208		ND	ND	very low
B209	0.94	very low	few[d]	very low
B210		very low–low	⅓	very low
B211	1.88	low	⅔	very low
B212		GM very low, sm low	¼	very low
B213	3.75	low–mod	⅔	very low
B214		GM mod, sm low	½	very low
B215	7.5	mod	⅔	low–mod
B216[e]		mod	⅔	low
B217[f]	15	mod	½	low–mod
B218[g]		mod	⅔	mod
B219[e]	30	GM mod, sm high	⅔	mod
B220		M mod, m high	⅔	low–mod
B221	60	GM high, sm mod	⅔	mod–high
B222		high	⅔	mod–high
B223	120	high	GM	high
B224		high	all	high

[a] Weight range 20.7–32.5, average 24.6 gm. Delay from initiation of pithing to first visualization of liver (LUL): range 65–125, average 78 seconds. Delay from completion of observations upon LUL to first visualization of LLL: range 15–30, average 20 seconds. Studies accomplished in July.

[b] All axial canaliculi delineated unless otherwise specified.

[c] Intensity on the whole somewhat lower in LLL than in LUL.

[d] c. ¼ delineated in LUL, occasional in LLL.

[e] 1 instance of intracellular vacuole formation, LLL.

[f] 1 imbibition cell, mod intensity, nucleus not visualized, LLL.

[g] 1 instance of intracellular vacuole formation, LUL.

largements of the axial canaliculi, and dilatations at the ends of their intercellular branches. As with fluorescein, these are interpreted as normal variants. Recognizable abnormalities of the canaliculi and hepatic cells ("sprouting", intracellular vacuole formation and imbibition cells) are at a minimum. Whenever delineated, even with extreme delicacy, the canaliculi always appear as a continuous system, without detectable interruptions in their course.

Studies with graded dosage of esculin are briefly summarized in Table II.

Some of the most informative pictures of the bile canaliculi are obtained at lower dosage, when the plasma fluorescence is suppressed. As with the fluorescent dyes, the caliber of the canaliculi is no larger with higher dosage than in a standard 30 mg. per kg. preparation, nor do they exhibit any other appreciable difference in character.

In all of these studies, for reasons previously discussed (1, 2), we have relied almost entirely upon evaluation of the initial status of the fluorescent images in frogs pithed at intervals following injection of the fluorescent compound. The sequence of events when the liver is held under continuous observation is illustrated by the protocol published elsewhere (1). However, one is fully justified in questioning the validity of information so obtained, and such studies have not been pursued further at this time. It should be mentioned that, in contrast to the findings with fluorescein, thioflavine S and vasoflavine, the frog does not absorb esculin from an aqueous environment in amounts sufficient to appear in the liver and to delineate the bile canaliculi (24 hours' exposure to a concentration of 1:50,000).

SUMMARY

Data are presented upon the sequence of events during the excretion of esculin, as observed *in vivo* in the frog's liver by the technic of fluorescence microscopy. Studies with graded dosage of the glycoside, over a very wide range, are likewise reported. With a dosage of 30 mg. per kg., esculin is cleared from the liver rather rapidly (c. 24 hours or less), at a rate comparable with that for fluorescein, and in sharp contrast to the prolonged excretion time for thioflavine S and vasoflavine (2 weeks and more).

LITERATURE CITED

1. GRAFFLIN, A. L. AND BAGLEY, E. H.: Studies of hepatic structure and function by fluorescence microscopy. Bull. Johns Hopkins Hosp., 1952, **90:** 395–437.
2. GRAFFLIN, A. L. AND CORDDRY, E. G.: In vivo studies of the frog's liver during the excretion of thioflavine S and vasoflavine. Bull. Johns Hopkins Hosp., 1954, **94:** 1–21.

THE APERIODIC BALLISTOCARDIOGRAPH*

S. A. TALBOT, D. C. DEUCHAR†, F. W. DAVIS, JR. AND W. R. SCARBOROUGH†

Department of Medicine, The Johns Hopkins University School of Medicine and Hospital

Received for publication November 13, 1953

An experimental and theoretical analysis of current methods of ballistocardiography (BCG) has been conducted in this department, and some results reported (1). A detailed account is in preparation. The purpose of this communication is to describe a promising principle and experimental system, which developed from these studies.

The BCG measuring systems associated with Starr, Nickerson and Dock all load the body by a heavy, constrained platform or a rigid table, so that the BCG movements of the body evoke spring and damping forces from the soft tissues of the dorsum of the subject. These forces create oscillations without cardiovascular meaning, whose frequencies fall in the midst of the BCG range. There results a serious distortion of phase and amplitude in all these records, of fundamentally the same character.

In an ideal system, as already stated by Nickerson (2) and Burger, *et al.* (3), the body would be free of all restoring or damping forces, as if floating in free space. The body's acceleration then would measure directly the force pattern transmitted to it from the cardiovascular system, assuming a unified body-mass (4).

Toward this ideal, Burger (3) has tested a damped suspended platform of such low period that the restoring forces and necessary damping are small. With period (3 sec.) near that of respiration, the subject must suspend breathing; which may considerably alter the wave form. Burger showed that the resulting *displacement BCG* depicts without distortion the internal *displacement* of heart and blood during the cardiac cycle. Wittern (4) independently has described a pendular bed of 2 sec. period, similar in principle. He points out that the *acceleration BCG* of such systems depicts the internal *forces* without distortion, and permits free breathing. Both these beds show limitations for high frequency components of the BCG, because of platform mass and damping.

We have sought the ideal of a dynamically free body by methods of flotation which require less platform mass, and being aperiodic need no damping. In the horizontal plane a bed of any liquid has these properties; mercury in particular

* This work was supported by a grant from the Life Insurance Medical Research Foundation, and (in part) by a grant (H-327) from the National Heart Institute, National Institutes of Health, Public Health Service.

† Fellow in Residence, Howard Hughes Foundation.

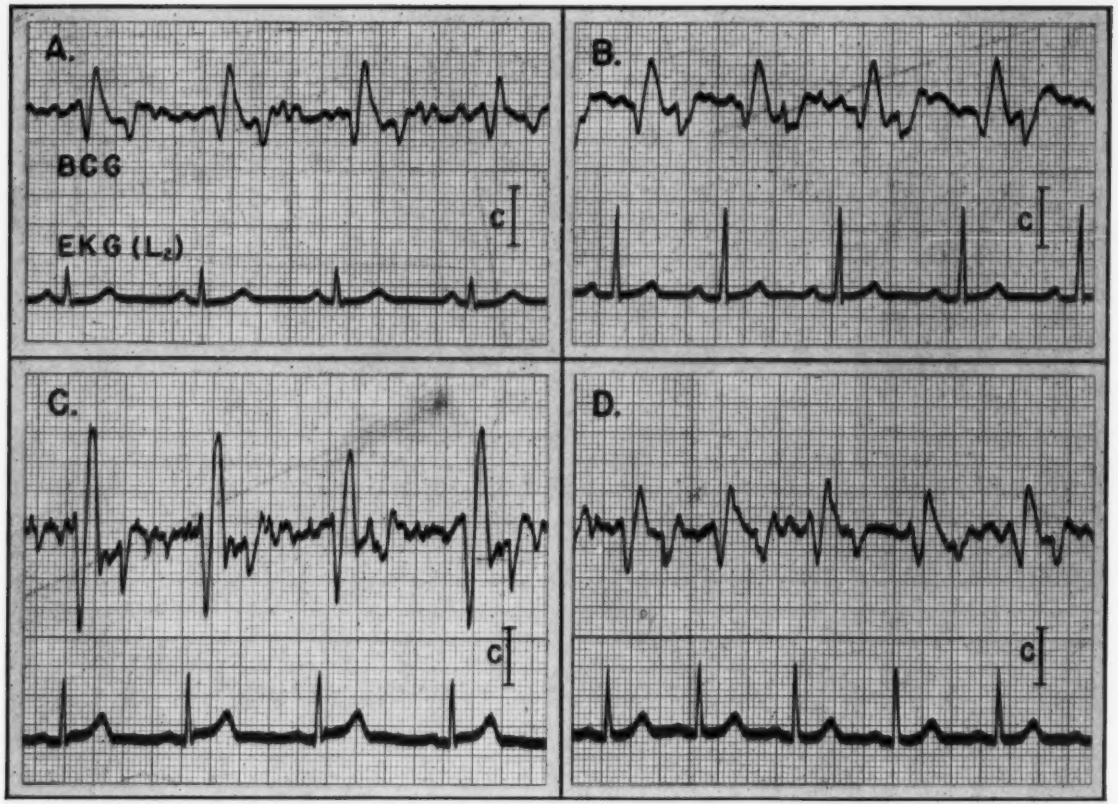
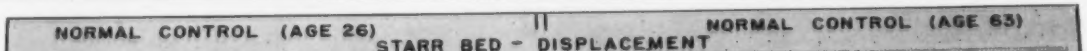


FIG. 1. Typical acceleration BCG records obtained from the mercury bed in four normal subjects, together with the simultaneously recorded electrocardiograms. (A) A man age 33 yrs. (B) A woman age 21 yrs. (C) A man age 33 yrs. (D) A woman age 23 yrs. Note the general resemblance of the form of the records. The calibration, marked C on the records, represents the deflection produced by 0.002 g = .8 in./sec.² acceleration, or 136 gm. force on a 150 lb. body.



simultaneously recorded electrocardiograms. (A) A man age 33 yrs. (B) A woman age 21 yrs. (C) A man age 33 yrs. (D) A woman age 23 yrs. Note the general resemblance of the form of the records. The calibration, marked C on the records, represents the deflection produced by 0.002 g = .8 in./sec.² acceleration, or 136 gm. force on a 150 lb. body.

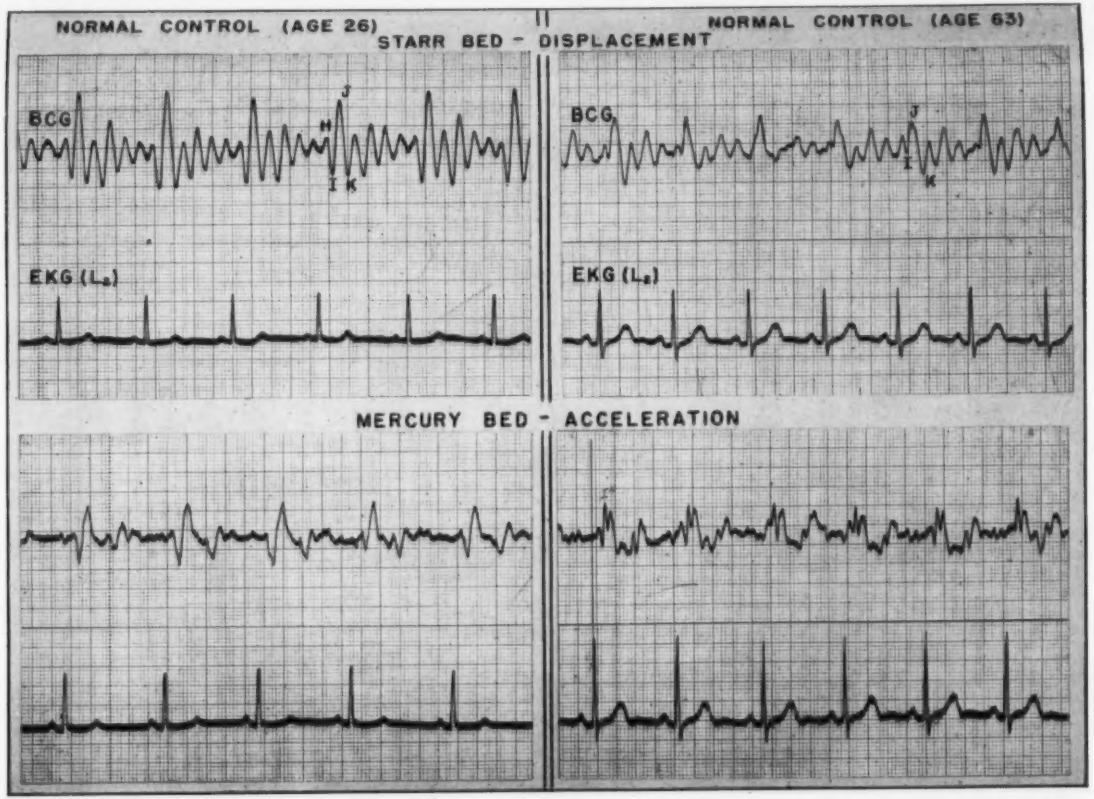


FIG. 2. Normal and abnormal mercury bed records together with the Starr bed records for the same subjects. Left —the records of a normal woman aged 26. Right—the records of a clinically normal man aged 63 with abnormal Starr bed record.

conserves support in the dorsal plane. While this fluid was chosen primarily to study the segmentation of the body in BCG motion, we will discuss here only the modification where a platform is added to average the output motions, as in current clinical ballistocardiography.

METHOD

The uniform support by a fluid permits a very light platform; here a plywood board weighing 11 lbs. In a long tank containing 375 lbs. of mercury (8 mm. deep) this platform with subject sinks 6 mm. Records were taken from shin or platform; a footboard can be used to improve transmission of high frequency. To interpret the BCG as force we have used an accelerometer, with seismic or internal reference to avoid high-frequency building vibrations. The strain-gauge type (Statham) with carrier amplification (Sanborn) gives acceptable signal-noise ratio. An electro-magnetic velocity-pickup is also acceptable.

The platform loaded with 150 lb. subject responds to a tap by a drift, on which appears a "swash" oscillation of 12 sec. period, too small to record by accelerometer. At high frequencies, the "corner" calculated for an 11 lb. platform is at 18 cps., so that components

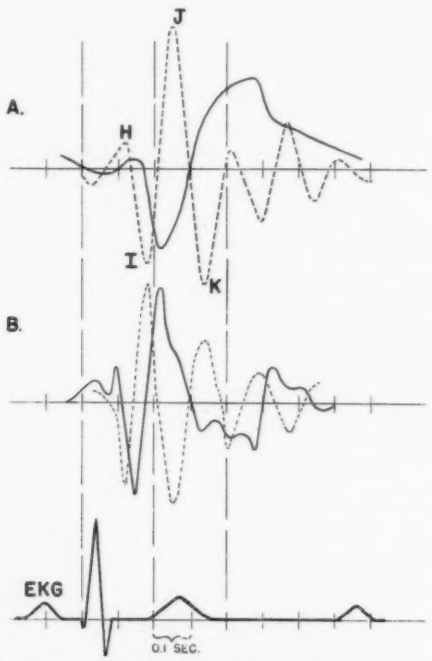


FIG. 3. A diagram to show the relationships between the electrocardiogram, the Starr-type or direct-body ballistocardiogram and the mercury bed record. The timing of the Starr-type records are average figures (5). Movements of the mercury bed platform are shown by the continuous line; A represents a displacement and B an acceleration record. Superimposed (broken line) are representations of a Starr-type record and its acceleration derivative, at A and B respectively. The differences in the timing and form of the waves can be clearly seen.

above this may be attenuated. The response from transducer to pen is flat to 40 cps. with sensitivity of 5 mm. per cm./sec.^{2} (approx. 5 mm./milli-g).

RESULTS

We have obtained records from 20 normal subjects (i.e., individuals without evidence of cardiovascular disease and with normal ballistocardiograms on the Starr-type bed). These records show a satisfactorily consistent pattern with easily recognizable waves occurring in all the subjects (Fig. 1). The timing also is consistent from individual to individual within physiological limits. In our group an early, small, headward wave of short duration (.04 sec.) occurs 0.07–0.11 seconds (mean 0.09 sec.) after the Q wave in lead II of the electrocardiogram; a large footward peak follows, 0.11–0.16 seconds after the Q (mean 0.14 sec.). The main headward peak (max. ampl. .003 g or 1 in./sec.^{2}) is next, occurring 0.18–0.24 seconds after the Q wave (mean 0.21 sec.). The succeeding part of the complex is somewhat variable in form, usually a segment somewhat footward of the base line, often containing two small waves. Following this is a fairly well-defined headward wave; this last wave occurs 0.48–0.58 seconds after the Q (mean 0.52 sec.). The time intervals noted do not correlate with the duration of the cardiac cycle.

A few subjects with abnormal ballistocardiograms as recorded on the Starr-type bed yield easily recognizable abnormal records on the mercury bed also (Fig. 2).

The record obtained from the mercury bed differs fundamentally from that obtained by the Starr bed and the direct-body method (Fig. 2) or the acceleration derivative of these. The relationships are illustrated diagrammatically in Figure 3. The main headward peak is located half way between the acceleration and displacement peaks of the Starr or Dock record. This shows the phase error of these systems (about 40 ms. for the J wave) compared with systems essentially aperiodic (without resonance) in the BCG spectrum. The main differences in form relations are in the JK segment and diastolic detail.

DISCUSSION

As mentioned above our immediate object was to achieve a system of frequency response having an improved range of linearity, by using a different mechanical principle. It is interesting to note that our records resemble those obtained by Wittern (4), and that displacement measurements of our platform (whether obtained by graphical integration of the acceleration records or electrical integration of the output of a velocity pickup) have the same general form as those published by Burger *et al.* Rappaport (6) also has set up a low-frequency suspended platform system and recorded its accelerations; the records so obtained are similar in form to ours. These resemblances would seem to provide experimental support for the validity of the analytical reasoning which led to the design of all these systems.

The reasons for using mercury have been mentioned; other liquids will do. We have taken records with an 8 lb. boat of kayak construction, sinking 3″ in water* with load. The acceleration BCG shows detailed similarity to records from the same two subjects on mercury. The main values of fluid support are (a) the extension of undistorted rendition well below breathing frequencies, confirming the existence of a major component of the displacement BCG at 1 cps., of sawtooth form (3); (b) the extension of undistorted rendition well above 20 cps. without physical constraints (4): a consideration important with heart disease. The initial headward wave is sharp enough to require a 30 cps. passband for resolution. The very light platform also reveals in many individuals consistent splitting in late systolic and early diastolic waves, apparently in the 30–40 cps. range of detail. These advantages of the principle of aperiodic support should also apply to air bags and other modes of realizing it.

Liquid supports furthermore have equally good response characteristics in the lateral (L-R) direction, for vector ballistocardiography in the frontal plane. While much freer from artefacts due to the support than with rigid tables or constrained platforms, such lateral BCG records still suffer from torsional admixture: the rolling of the body due to level of impact above the level of support. We are therefore not presenting our data on the lateral BCG of fluid beds at this time.

CONCLUSION

It is thought that these methods give a much less distorted picture of the ballistocardiographic forces than the earlier systems. By these aperiodic systems we are getting closer to the ultimate generator of the forces in which we are interested. We should therefore be able to achieve a more satisfactory correlation of ballistocardiographic form with physiological factors.

Further studies with the fluid bed systems here described are being conducted, whilst work is simultaneously being done on the development of more practicable aperiodic systems for achieving the same results.

Advances in understanding and interpretation of clinical records may be expected from the increase of reliable detail for individual subjects, which results from this principle of aperiodic supports.

SUMMARY

The undistorted frequency spectrum of ballistocardiography in the head-foot direction may be extended significantly by using a light platform or boat on liquid. The acceleration BCG of normals from such a support shows consistent and characteristic features in considerable detail. The principle of aperiodic support is that approached by the pendular beds of Burger and Wittern.

* Dock has recorded the ballistic displacement of the sternum, using a subject immersed in water (7).

REFERENCES

1. HARRISON, W. K. AND TALBOT, S. A.: Dynamical description of the ballistocardiograph. Fed. Proc., 1953, **12:** 62.
2. NICKERSON, J. L. AND CURTIS, H. J.: The design of the ballistocardiograph. Am. J. Physiol., 1944, **142:** 1.
3. BURGER, H. C., NOORDERGRAAF, A. AND VERHAGEN, A. M. W.: Physical basis of the low-frequency ballistocardiograph. Am. Heart J., 1953, **46:** 71.
4. WITTERN, W. W. v.: Ballistocardiography with elimination of the influence of the vibration properties of the body. Am. Heart J., 1953, **46:** 705.
5. SCARBOROUGH, W. R., ET AL.: A review of ballistocardiography. Am. Heart J., 1952, **44:** 910.
6. RAPPAPORT, M. B.: Personal communication, 1953.
7. DOCK, W., MANDELBAUM, H. AND MANDELBAUM, R. A.: *Ballistocardiography*. The C. V. Mosby Co., St. Louis, Missouri, 1953, pg. 47.

OBSERVATIONS ON THE ETIOLOGY OF RETROLENTAL FIBROPLASIA

HARRY H. GORDON,[1] LULA LUBCHENCO[2] AND IVAN HIX[3]

Departments of Pediatrics and Ophthalmology of the University of Colorado, Sinai Hospital, Baltimore, and Department of Pediatrics, the Johns Hopkins University School of Medicine

Received for publication November 17, 1953

In a series of papers published between 1942 and 1945, Terry (1) gave the name retrolental fibroplasia to a condition in prematurely born infants characterized by blindness and the formation of an opaque membrane behind the lens. There was subsequently a striking increase in the number of cases seen so that it became in some regions the major cause of blindness in pre-school children. It is the purpose of this paper to discuss the problem of etiology.

In 1948, Owens and Owens (2) reported the results of repeated ophthalmoscopic examinations of 214 premature infants admitted to the Johns Hopkins Hospital either by birth or transfer from place of birth. In their classic description they characterized the disease as affecting primarily the blood vessels of the retina, a concept which became the basis of virtually all current studies. They classified the ophthalmoscopic findings in four stages, the first two consisting of dilatation and tortuosity of retinal vessels, followed by retinal edema and hemorrhages; at this point the disease was still completely reversible. In the next stages, the process went on to neovascularization, detachment of the retina and formation of a retinal membrane behind the lens.

More recently, with Friedenwald (3) they have presented an elegant description of the early histological changes. These are found in the nerve fiber layer of the retina, where in localized areas, the capillary endothelium shows marked proliferation with varicose dilatations and abnormal budding of the vessels. No infiltration with polymorphonuclear or inflammatory mononuclear cells is found in the retina, the disease appearing as an abnormal growth of capillaries which at times form whorls resembling glomerular tufts. Similar changes have been reported by Reese, Blodi and Locke (4), and by Heath (5).

The demonstration that the fundi of small premature infants could be examined ophthalmoscopically led to quick confirmation of the Owens' findings in many centers, and indicated that the term retrolental fibroplasia was as inadequate a term for this disease as is mitral stenosis for rheumatic carditis. The

Presented in part before the Johns Hopkins Medical and Surgical Association, February 27, 1953.

This study was aided by grants from the National Society for the Prevention of Blindness and the Association for the Aid of Crippled Children.

[1] Baltimore, Maryland.

[2] Denver, Col.

description of the reversible stages led to a sharp increase of (a) interest in making the diagnosis, (b) speculation concerning and study of possible factors in etiology and treatment, and (c) confusion as to incidence because of variations in quality and interpretations of the ophthalmoscopic examinations, and in duration of follow-up. There is much less confusion, however, about incidence of residual lesions which range from retrolental membranes covering the pupillary area to small masses of opaque tissue in the periphery of the fundus without visible detachment of the retina.

We should like at this point to review experiences with the disease at the Colorado General Hospital in Denver. A special program for the care of premature infants was started in May 1947, but small infants born elsewhere were not admitted to the Premature Center until February 1948.* During the next two years we saw only scattered patients with residua of retrolental fibroplasia, the only stages of the disease which we could have recognized with the type of follow-up then being given. In 1950, however, there was a sharp rise in incidence, and at one time during the summer of 1950, seven of eleven infants then in the nursery had severe residual lesions. It was obviously time to take stock of how the care given to the premature infants in the nurseries at Denver differed from that which one of us had supervised at the New York Hospital ten years earlier. The feeding was essentially the same: moderate total intakes of partially skimmed cows' milk with added carbohydrate, feeding mixtures that Powers and Park had used for years at New Haven Hospital and Harriet Lane Home. The use of water miscible vitamins did not appear to be responsible, because after our first cases, the survey of Kinsey and Zacharias (6) had led us back to percomorph oil. Iron medication was not being given, and transfusions were being used only for anemia, actually a restriction in use as compared with our practice ten years earlier. An obvious difference in care however was in the use of oxygen; this had been mentioned by Kinsey and Zacharias (6) but had not been pursued in later studies (7). Oxygen was being used extensively in the Denver nurseries as in most modern premature units; as a matter of fact, the nurseries had been transferred in April 1950 to new quarters in which the piping in of oxygen from a central supply had made it as easily available as water from a faucet.

Some reasons for the extensive use of oxygen in the care of premature infants are worth noting. With decreases in mortality from infection, there has been increasing emphasis on deaths in the first days of life because of respiratory failure. Small premature infants have marked handicaps in respiration. They may have poor gag and cough reflexes, under-developed capillaries in the lungs, medulla and other tissues, sparse elastic tissue in the lungs. They are more seriously damaged by perinatal factors which lead to anoxia. They have spon-

*The Premature Infant Center was established at the Colorado General Hospital in cooperation with the Colorado State Health Department and the U. S. Children's Bureau.

taneous attacks of apnea, and irregular respiration in room air, the latter two items being considered manifestations of defective neural or humoral control of respiration. These considerations and the increased availability of incubators had led to an extensive routine use of oxygen in the care of premature infants during the first few days of life. Since no information is available concerning optimal environmental concentrations or duration of oxygen therapy, the use in Denver as well as in most nurseries had been for the most part unscrutinized. We found, on checking, that the infants were being kept in incubators in which the flow of oxygen probably resulted in concentrations well over 50 to 60 per cent. Steps were taken to educate the nursing and medical staffs that infants could be raised successfully with initial incubator concentrations of oxygen of only 30 to 40 per cent. It was found that the infants could be weaned more quickly altogether to room air, so that the duration of oxygen therapy was also decreased. Furthermore, there was virtually no retrolental fibroplasia in the nursery during the first months after the change, and any consideration of now exposing alternate infants to high concentrations of oxygen was dropped. The results of a two year period of restriction of oxygen are now available for comparison with the previous periods.

For purposes of analysis, the infants have been divided into four groups. During Periods I and II, oxygen therapy was in general and as has been unfortunately the custom in most hospitals, unscrutinized use; that is, measurements of concentrations in the environment were unsystematically made and rarely recorded. During Period I, from July 1947 to May 1950, the infants were kept for the most part in incubators in which the lids had to be opened to service the baby. Furthermore, the transfer of flow meters from tank to tank required forceful use of wrenches with resultant inaccuracy of the flow meters. The resultant irregularities in concentrations of oxygen in incubators have been well described (8). We have designated this first period as one of unscrutinized oxygen therapy which was probably moderate because of the type of incubators used. In Period II, of five months, more efficient incubators with portholes through which service could be given to the babies were used and oxygen was more easily available because it was piped in, thus circumventing need for changes in tanks by nursery personnel. This period we have designated as one of unscrutinized high oxygen therapy since scattered analyses showed concentrations of 60 per cent and over in both the more and less efficient incubators. Period III, of only three months is designated as a transition period in which at first a few and then more infants received less oxygen than previously while the staff was gaining confidence that concentrations of 30 to 40 per cent would not necessarily lessen chances of survival of the infants. This transition period was required to maintain morale since one of the primary purposes of the Premature Center had been to teach respect for individuality of infants, and this had to be based on respect for the individuality and intelligence of the personnel

TABLE I

Distribution of Birth Weights of 211 Surviving Premature Infants Followed for Residual Lesions of Retrolental Fibroplasia

Period	I		II		III		IV	
Date	7/1/47–5/1/50		5/1/50–10/1/50		10/1/50–1/1/51		1/1/51–1/1/53	
Oxygen therapy	Unscrutinized				Scrutinized			
	Moderate		High		Transitional		Restricted	
Birth weight, grams	No.	%	No.	%	No.	%	No.	%
<1000	9	11	3	15	3	21	12	12
1001–1250	23	29	9	45	5	36	38	39
1251–1500	48	60	8	40	6	43	47	48
Total	80		20		14		97	

assigned to the nursery. Period IV is a two year period in which concentrations of oxygen were restricted to 30–40 per cent and in which the duration of oxygen therapy was also decreased. Infants are included in this study only if adequate information is available concerning the status of their eyes for periods of at least three months following birth; in the great majority this follow-up period was six months or longer. During Period I, ophthalmoscopic examinations were not made in all infants so that it is probable that some residual scars of the retina were missed. Such less obvious lesions are included in the subsequent periods when expert ophthalmoscopic examinations were available for all infants.

In Table I, the distribution of birth weights is given for 211 infants weighing less than 1.5 kg. who survived and were followed. It is seen that in the two periods crucial for the analysis of the effect of restriction of oxygen therapy, i.e. Periods II and IV, the proportions of infants in the three birth weight groups are essentially the same. This permits an over-all comparison of the incidence of retrolental fibroplasia which has been found in most series to be related inversely to birth weight (9).

In Table II, the incidence of residual lesions and of retrolental membranes is presented. During Period I, residual lesions were found in 15 per cent and membranes in 10 per cent of the 80 infants followed. During Period II, the incidence of residual lesions increased to 9 or 45 per cent, and 7 or 35 per cent had membranes. Restriction of oxygen over a two year period led to an incidence of only 8 per cent of residua in 97 infants and only 2 had membranes.

Although the original plan for keeping alternate infants in high or low oxygen was not followed, the results collected over a two year period in which 97 small infants were followed carefully stands as strong supporting evidence for the conclusion that the administration of oxygen in high concentrations has been

TABLE II

Incidence of Residual Lesions of Retrolental Fibroplasia

PERIOD	I			II			III			IV		
Oxygen therapy	Unscrutinized						Scrutinized					
	Moderate			High			Transitional			Restricted		
Birth weight, grams	Infants, No.	Residua		Infants, No.	Residua		Infants, No.	Residua		Infants, No.	Residua	
		No.	%		No.	%		No.	%		No.	%
<1000	9	3	33	3	2	67	3	1	33	12	2	16
1001–1250	23	5	22	9	4	44	5	3	60	38	6	16
1251–1500	48	4	8	8	3	38	6	0	0	47	0	0
Total	80	12	15	20	9	45	14	4	29	97	8	8
Membranes		8	10		7	35		3	21		2	2

Calculation of the statistical significance of the results shows the following:

COMPARISON OF	RESIDUA	MEMBRANES
Periods II and IV	$Chi^2 = 15.21$ P < 0.001	$Chi^2 = 20.95$ P < 0.001
Periods I and IV	$Chi^2 = 1.38$ P 0.2–0.3	$Chi^2 = 3.79$ P 0.05

the main cause of this disease. Campbell (10) and Ryan (11) in Australia, Crosse and Evans (12) and Jefferson (13) in England have published similar conclusions from studies which have the same defects as the Denver study, i.e. lack of precision and lack of alternate controls, but have an added defect that at the time of publication they had not run as long.

These clinical observations serve to corroborate the careful studies of Patz and his coworkers (14). These investigators have published a study in which alternate infants were assigned to high or low oxygen and followed with ophthalmoscopic examinations for at least six months. Oxygen concentrations were measured three times daily and flows adjusted to maintain the desired concentrations. Of a total of 76 babies, all weighing less than 1.5 kg. at birth, eight were dropped because O^2 concentrations were not maintained and three failed to return for follow-ups. In the group of 28 infants kept in 65 to 70 per cent oxygen for 4–7 weeks, 11 or 40 per cent showed normal retinae throughout and 7 or 25 per cent showed stages III or IV of retrolental fibroplasia, i.e., changes which included detachment of the retina. In 37 infants who received minimal oxygen, never more than 40 per cent, and from one day to two weeks, 84 per cent had normal retinae and none developed residual changes. During a second year of study these results were confirmed and the total figures are now as follows: in low oxygen, 60 infants, only 1 of whom showed a residual lesion; in high oxygen, 60 infants, 12 of whom showed residua (15).

Exposure of newborn animals to high oxygen has yielded very strong supporting evidence. Gyllensten and Hellström (16) showed that intermittent ex-

posure of newborn mice to 100% oxygen for 1 to 3 weeks resulted in an ocular disease characterized by hemorrhages into the vitreous body, hyperplasia of the tunica vasculosa lentis, and detachment of the retina. Ashton and his co-workers (17) have recently reported that placing newborn kittens in high oxygen stops normal vascularization of the retina, and that when growth of capillaries is resumed on removal from oxygen it is disorderly. Finally, Patz and his co-workers (18) have used high oxygen to produce in newborn rats, mice, cats and dogs ocular lesions which include not only those found by Gyllensten and Hellström but the endothelial nodules in the retina described by Friedenwald and the Owens as characteristic of the early human disease.

Patz and his co-workers have thus shown conclusively both in infants and newborn animals that the retinal lesions of retrolental fibroplasia can be caused by the administration of high oxygen. The additional clinical and animal studies cited can be accepted as adequate corroboration.

One needs to consider two additional topics: (a) the effect of lowering environmental oxygen concentrations on survival of small premature infants and (b) whether excessive oxygen is the whole cause of this disease.

In Table III are presented survival rates for infants whose birth weights were less than 1.5 kg. and who were admitted to the University of Colorado Premature Infant Center either from the delivery rooms of the Colorado General Hospital or after birth in other institutions, at home, or while enroute to the hospital. Because small infants born at other institutions would ordinarily have survived the first 24 or 48 hours before transfer was considered, and because mortality rates are highest during this period, the "outborn" infants represent a selected group whose survival rates are, as expected, higher than those of the small infants born in the Colorado General Hospital. For each group it is seen, however, that the survival rates of 43 and 72 per cent respectively, during Period IV of restricted oxygen are at least as high as those in the other periods. Under the conditions of close medical and nursing supervision given infants at the premature center the restriction of oxygen was thus associated with no increase in mortality and with a significant decrease in residual lesions of retrolental fibroplasia.

The obvious importance of high oxygen should not exclude consideration of other etiologic factors both in the host and environment which may be interrelated. As an example of the former, one recognizes the disease as primarily one of smaller premature infants, usually weighing less than 1.5 kg. at birth. Vascularization of the retina occurs during the 4th to 8th month of fetal life (19). Just as one is more apt to find scorbutic or rachitic changes in rapidly growing bones, so it is during a period of rapid growth of retinal capillaries that disorderly growth may occur from exposure to a noxious agent. Although the disease has been reported occasionally in larger premature infants, it is recognized that birth weight is only a rough guide to maturity and is accepted

TABLE III

Survival Rates of Premature Infants Weighing Less than 1.5 kg. at Birth, 1 July 1947–1 January 1953

PERIOD	I			II			III			IV		
Oxygen therapy	Unscrutinized						Scrutinized					
	Moderate			High			Transitional			Restricted		
Birth weight, grams	Adm., no.	Lived, no.	Survived, %	Adm., no.	Lived, no.	Survived, %	Adm., no.	Lived, no.	Survived, %	Adm., no.	Lived, no.	Survived, %
Born at Colorado G. H.												
Less than 1000	19	4	21	4	0	0	1	0	0	13	1	8
1001–1250	11	4	37	3	2	67	0	0	—	10	3	30
1251–1500	11	8	73	3	1	33	0	0	—	17	13	76
	41	16	39	10	3	30	1	0	0	40	17	43
"Outborn"												
Less than 1000	34	5	15	8	3	38	6	3	50	27	11	41
1001–1250	37	21	57	8	7	88	5	5	100	55	40	73
1251–1500	62	47	76	9	8	89	10	7	70	54	47	87
	133	73	55	25	18	72	21	15	71	136	98	72
Grand totals	174	89	51	35	21	60	22	15	68	176	115	65

rigidly for statistical purposes because of the inaccuracies in determining gestational age. Data collected by Silverman and his associates suggest that at any given birth weight, infants of lower gestational age and at any given gestational age, infants of lower birth weight, develop the disease more frequently (20). Finally, there are available histologic specimens of the eyes of stillborn premature infants which show endothelial nodules in the retinae similar to those described by Friedenwald and the Owens (3). Reese (4) has reported two pairs of such eyes and Owens has one pair (21). Griffiths (22) has found the early lesion in the eyes of a premature infant who lived only 9 hours. The number of stillborn premature infants whose eyes have had careful histologic examinations is too small to warrant assured talk about the significance of these findings i.e., whether they represent a congenital anlage or the effect of a prenatal noxious agent.

Although the evidence points conclusively to high oxygen as the major environmental cause, retrolental fibroplasia has occurred in liveborn infants who received no or minimal additional oxygen after birth. The Owens have one patient who was reared at home with no added oxygen and who developed the full-blown disease (21). There are three infants in the Denver series who received added oxygen for less than 3 days. Bembridge (23) mentions one infant and Coxon (24) two, who developed the disease with no oxygen added to their

environment, and Dancis and his co-workers state that retrolental fibroplasia developed in some infants who were never placed in incubators (25). Campbell, Ryan and Patz all point out, however, that even in room air the oxygen saturation of the infant's blood rises to levels much higher than existed in utero. Actually, as soon as one considers the striking changes in environment which take place at birth, one need not be surprised that growth, in this instance of retinal capillaries, may become disorderly. Our methods of giving foodstuffs which include oxygen are a far cry both qualitatively and quantitatively from the maternal venous blood which nourishes the infant in utero. Mitigation of the degree of prematurity, i.e. keeping the baby in utero for as long as possible, constitutes an unquestioned method of reducing both the incidence of retrolental fibroplasia and mortality.

There are two reports from centers in which the presumed use of high oxygen was not associated with an increased incidence of retrolental fibroplasia. A large series has been reported from the Charity Hospital in New Orleans where incubators were used with flows of 4 liters per minute presumed to give environmental concentrations of 50 per cent oxygen (26). Since the incubators were of the type in which wide opening of the incubator was necessary in order to service the infants, and since the large average daily census of approximately one hundred infants accentuates nursing shortages it is possible that the infants were not exposed to as high concentrations as thought. In the second large series, reported from Paris, the analysis is not broken down into weight groups and all infants under 1800 grams are included (27). It is difficult to evaluate these data.

Anoxia has been suggested by one investigator (8b) who reported that taking the babies out of high oxygen led to quick development of vascular changes which could be reversed by returning the infants to high oxygen. Since regression of early changes has occurred in a majority of instances without replacement in oxygen, since in some infants regression has failed to occur when infants were returned to oxygen, since later stages have developed in infants while they were continuously in high oxygen (14), since the lesion has been produced experimentally in rats and mice while they were continuously in high oxygen (18), it is difficult to consider that withdrawal of infants from oxygen can be primarily responsible for the disease. Actually, the basic mechanisms responsible for the pathogenesis of the vascular changes are still obscure, but the experimental production of the disease in a variety of animals supplies material for controlled studies to determine whether sudden removal from oxygen aggravates a vascular disturbance caused by administration of excessive amounts of oxygen. The investigator (8b) who suggested sudden anoxia as a cause for the disease has also suggested that the way to prevent the disease is to avoid putting the infants in high oxygen.

Feeding cows' milk mixtures which are higher in electrolytes than human

milk has been suggested on several occasions as a possible cause of retrolental fibroplasia. Hepner and Krause (28) noted that two premature infants who were fed amounts of cows' milk greater than is generally accepted as desirable and containing added sodium chloride developed retinal edema and detachment. Although no satisfactory clinical or animal studies have been presented in support of this concept, yet it is possible that sudden expansion of blood volume by overfeeding or overtransfusion may adversely affect the capillary development in the retina particularly if this has already been made disorderly by the administration of high oxygen. It has also been suggested that increased extracellular concentrations of sodium might adversely affect intracellular metabolism in the retina (29).

A word should be said about the early suggestion of the Owens (30) that a lack of tocopherol in the cows' milk mixtures fed premature infants and their poor fat absorption might be responsible for retrolental fibroplasia. Subsequent studies have shown that premature infants on these mixtures have low plasma tocopherol (31), and that the susceptibility of their red blood cells to hydrogen peroxide is reversed by administration of tocopherol (32), thus confirming the Owens' suggestion that these diets are deficient in tocopherol. Since tocopherol is an antioxidant and since the administration of oxygen has been proven responsible for the disease, one can speculate that tocopherol might have some protective effect provided the amount of oxygen administered was not beyond this suggested protective effect. The unconfirmed beneficial effects reported by the Owens' both in a small alternate series and then in a larger uncontrolled group (30) might be confirmed if one studied only patients who were receiving minimal amounts of oxygen.

We recently discussed the problem with Dr. Park who is concerned with the effects of rapid growth not only on bones but on other tissues, and so our thinking runs naturally to analogies with rickets. Rickets is a disease in which lack of dietary vitamin D is the most common cause, but rate of growth, variations in calcium and phosphorus content of the diet, fat absorption, renal function, citrate metabolism and utilization of the vitamin may at times play roles. Retrolental fibroplasia is a disease in which high environmental oxygen is most important, but in which the condition of the capillaries at birth, rate of growth, or dietary components like electrolyte or tocopherol content may play roles. How these factors may affect capillary growth is probably an exciting chapter on its own.

SUMMARY AND CONCLUSIONS

1. The incidence of residual lesions of retrolental fibroplasia in 211 prematurely born infants weighing less than 1.5 kg. at birth, was analyzed in relation to oxygen administration.

2. Restriction of oxygen led to a marked drop in incidence over a two year

period in both residual lesions and membranes as compared with a previous period of unscrutinized high oxygen therapy.

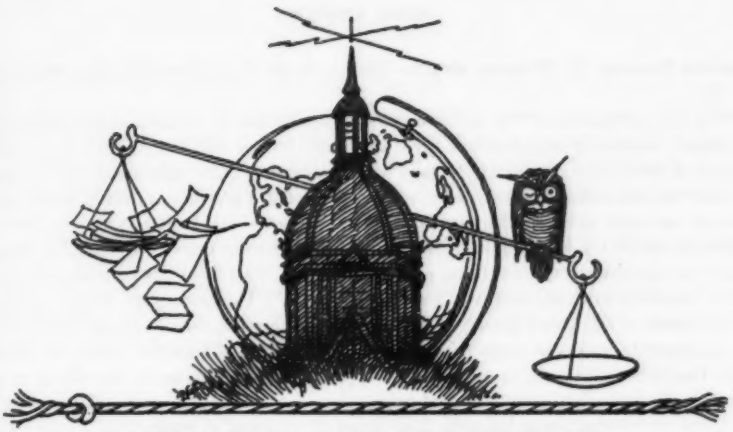
3. Survival rates were not adversely affected by restriction of oxygen.

4. A discussion is given of factors other than high oxygen which may contribute to the etiology of the disease.

BIBLIOGRAPHY

1. a) TERRY, T. L.: Extreme prematurity and fibroplastic overgrowth of persistent vascular sheath behind each crystalline lens. Am. J. Ophth. 1942, **25:** 203.
   b) TERRY, T. L.: Ocular maldevelopment in extremely premature infants—Retrolental fibroplasia. VI. General considerations. J. Am. Med. Assn., 1945, **128:** 582.
2. a) OWENS, W. C. AND OWENS, E. U.: Retrolental fibroplasia in premature infants. Tr. Am. Acad. Ophth. 1948, **53:** 19.
   b) OWENS, W. C. AND OWENS, E. U.: Retrolental fibroplasia in premature infants. Am. J. Ophth. 1949, **32:** 1.
3. FRIEDENWALD, J. S., OWENS, W. C. AND OWENS, E. U.: Retrolental fibroplasia in premature infants. III. The pathology of the disease. Tr. Am. Ophth. Soc. 1952, **49:** 207.
4. REESE, A. B., BLODI, F. C. AND LOCKE, J. C.: The pathology of early retrolental fibroplasia; with an analysis of the histologic findings in the eyes of newborn and stillborn infants. Am. J. Ophth. 1952, **35:** 1407.
5. HEATH, P.: Retinopathy of prematurity: A discussion of histopathologic characteristics. New York State J. Med. 1952, **52:** 2875.
6. KINSEY, V. E. AND ZACHARIAS, L.: Retrolental fibroplasia: Incidence in different localities in recent years and a correlation of the incidence with treatment given the infants. J. Am. Med. Assn., 1949, **139:** 572.
7. KINSEY, V. E. AND CHISHOLM, J. F. JR.: Retrolental fibroplasia: Evaluation of several changes in dietary supplements of premature infants with respect to incidence of the disease. Am. J. Ophth. 1951, **34:** 1259.
8. a) DANCIS, J. AND CARDULLO, H. M.: Incubator care of the premature infant. Pediatrics 1951, **6:** 432–441.
   b) SZEWCZYK, T. S.: Retrolental fibroplasia: Etiology and prophylaxis. Am. J. Ophth. 1952, **35:** 301.
9. ZACHARIAS, L.: Retrolental fibroplasia: A survey. Am. J. Ophth. 1952, **35:** 1426.
10. CAMPBELL, K.: Intensive oxygen therapy as a possible cause of retrolental fibroplasia Med. J. Australia 1951, **2:** 48–50.
11. RYAN, H.: Retrolental fibroplasia. Am. J. Ophth. 1952, **35:** 329–342.
12. CROSSE, V. M. AND EVANS, P. J.: Prevention of retrolental fibroplasia. Arch. Ophth. 1952, **48:** 83–87.
13. JEFFERSON, E.: Retrolental fibroplasia. Arch. Dis. Child. 1952, **27:** 329.
14. PATZ, A., HOECK, L. E. AND DE LA CRUZ, E.: Studies on the effect of high oxygen administration in retrolental fibroplasia: I. Nursery observations. Am. J. Ophth. 1952, **35:** 1248.
15. PATZ, A.: Oxygen studies in retrolental fibroplasia. III. Results of a 5-year nursery study and resumé of animal experiments. (To be published).
16. GYLLENSTEN, L. J. AND HELLSTRÖM, B. E.: Retrolental fibroplasia—animal experiments Acta Paediat. 1952, **41:** 577.
17. ASHTON, N., WARD, B. AND SERPELL, G.: Role of oxygen in genesis of retrolental fibroplasia. Brit. J. Ophth. 1953, **37:** 513.

18. Patz, A., Eastham, A. B., Higgenbotham, D. and Klak, T.: Oxygen studies in retrolental fibroplasia in experimental animals. Am. J. Ophth. (In Press).
19. Mann, I. C.: *The Development of the Human Eye.* Cambridge University Press, New York, 1928.
20. Silverman, W. A., Blodi, F. C., Locke, J. C., Day, R. L. and Reese, A. B.: Incidence of retrolental fibroplasia in a New York nursery. Arch. Ophth. 1952, **48:** 698.
21. Owens, W. C., Personal communication.
22. Griffiths, R., Personal communication.
23. Bembridge, B. A., Coxon, M., Houlton, A. C. L., Jackson, C. R. and Smallpiece, V.: Retrolental fibroplasia, a problem of prematurity. Brit. Med. J. 1952, **4760:** 675.
24. Coxon, M. W.: Cases of retrolental fibroplasia. Tr. Ophth. Soc. of U. Kingdom. 1951, **71:** 591.
25. Dancis, J., Lewis, J. M. and Guy, L. P.: Retrolental fibroplasia: A statistical study with particular reference to the effect of vitamin A. New England J. Med. 1951, **245:** 402.
26. Exline, A. L. Jr. and Harrington, M. R.: Retrolental fibroplasia. J. Ped. 1951, **38:** 1.
27. Lelong, M., Rossier, A., Fontaine, M., Lemasson, C., Michelin et Audibert, J. Sur la retinopathie des prématurés. Arch. Franc. de Pediatrie, 1952, **9:** 898.
28. Hepner, W. R. Jr., and Krause, A. C.: Retrolental fibroplasia: Clinical observations. Pediatrics 1952, **10:** 433.
29. Darrow, D. C.: Comment on paper by Hepner, W. R. Jr.: Am. J. Dis. Child. 1952, **84:** 750.
30. Owens, W. C. and Owens, E. U.: Retrolental fibroplasia in premature infants: Studies on the prophylaxis of the disease: The use of alpha-tocopherol acetate. Am. J. Ophth. 1949, **32:** 1631.
31. Wright, S. W., Filer, L. J. Jr. and Mason, K. E.: Vitamin E blood levels in premature and full term infants. Pediatrics 1951, **7:** 386.
32. Gordon, H. H. and de Metry, J. P.: Hemolysis in hydrogen peroxide of erythrocytes of premature infants. Effect of alpha tocopherol. Proc. Soc. Exp. Biol. and Med. 1952, **79:** 446.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Managing Your Coronary. By WILLIAM A. BRAMS. 158 pp., $2.95. J. B. Lippincott Co., Philadelphia, Pa.

The book was written to tell patients, relatives, and friends what the author concluded they should know about a "coronary." The author, a recognized authority on the subject, collected over many years of practice, pertinent questions asked by interested laymen. The book is an attempt to answer these questions.

Written in simple easy style it accomplishes this purpose very well. It is neither too technical nor breezy. The author has wisely included case histories with an optimistic flavor so as not to provoke or increase unnecessarily the layman's anxiety. The book should make patients consult their physicians more intelligently rather than tempt them to treat themselves.

BENJAMIN M. BAKER

A Collection of Urogenital Drawings. By WILLIAM P. DIDUSCH. 222 pp. American Cystoscope Makers, New York.

William P. Didusch for years has been and continues to be the foremost illustrator in the field of urology. These collected drawings, well over 250 in number and many published here for the first time, clearly illustrate why. Each accurately (and beautifully) portrays some phase of urology whether it be normal anatomy, a congenital anomaly, a pathological state, or an instrumental or surgical maneuver. Perhaps what appeals most to this reviewer is the accuracy of these drawings, an accuracy made possible not only by the keenness of the observer and his ability to put it down on paper, but also by the remarkable breadth of his urologic experience. For those interested in teaching urology, whether to the undergraduate or graduate, this volume serves admirably in illustrating the subjects at hand. The pictures explain themselves.

W. W. SCOTT

Bacterial Genetics. By WERNER BRAUN. 238 pp., $6.50. W. B. Saunders Co., Philadelphia, Pa.

With the recognition of the many metabolic similarities of unicellular and multicellular organisms, increasing attention has been shown the former and has resulted in the uniting of many of the basic disciplines of biology with bacteriology. One outcome of this integration has been the rapid development of the subject of bacterial genetics, a field in which striking progress has been made in the past decade. As a result thereof, there has been need for a volume in which the important advances in knowledge were drawn together and placed in proper perspective with information previously extant. This need has been filled admirably by Dr. Braun in his book entitled "Bacterial Genetics". The monograph begins with a simple treatment of the classic principles of genetics and proceeds then to a lucid explanation of the experimental evidence supporting the mutational basis of bacterial variation. In subsequent chapters it deals with representative types of bacterial mutants, the effects of mutation and of adaptation upon bacterial populations and the several recognized mechanisms of genetic recombination in bacteria. The material reviewed is drawn from a wide range of sources and the bibliographic footnotes provide the reader with a useful array of the more important references pertaining to the topics discussed. Though the author's treatment of the subjects of bacterial colonial morphology, antigenic structure and virulence is at times somewhat at variance with views held by the reviewer, differences in emphasis may result in part from the fact that each has been concerned in the main with the study of different bacterial species. This volume represents a significant contribution to the literature of biology and can be read with interest and profit by all concerned in any fashion with the nature, behavior and interrelationships of living organisms.

ROBERT AUSTRIAN

Bakteriologische Nährböden. By LOTHAR HALLMANN. 252 pp., Ganzleinen DM 19.80. Georg Thieme Verlag, Stuttgart, Germany.

This volume is designed as a companion to an earlier publication entitled "Bakteriologie und Serologie" by the same author and as such is intended to be a practical manual for use in the preparation of media for the medical bacteriology laboratory. The initial chapter is devoted to certain biochemical activities of bacteria detectable through the use of suitable media and reagents and to the basic ingredients of media and their preparation in the laboratory. There follow a section on reagents and thereafter fourteen chapters devoted to a variety of special media including a large number of those used extensively in diagnostic bacteriology in this country. Many of the formulae included, however, may be of limited interest to workers in American laboratories, where, as pointed out by the author, commercially prepared dehydrated media are more widely employed than in Germany. The utility of this work by itself is lessened to some extent by the paucity of comment concerning the relative merits of different media designed for similar purposes, by scant reference to the role of antibiotics in the medical bacteriology laboratory and by the incompleteness of its indices, such subjects as media for the *Neisseria*, etc., being omitted therefrom though included in the text. The book is provided with a washable cover but its tabular marginal indentations seem less durable.

ROBERT AUSTRIAN

Einführung in die Innere Medizin, 5th edition. By HANS JULIUS WOLF. 714 pp., Ganzleinen DM 35. Georg Thieme Verlag, Stuttgart, Germany.

Here is a text-book of medicine which fulfils to an unusual degree the requirements of students, practitioners and teachers. The style is fresh and vivid with parenthetical practical

warnings inserted throughout the discussion. The use of small print allows the author to review briefly important biochemical and physiological concepts in connection with the disease being discussed. In the section on diabetes there is a short discussion of intermediate carbohydrate metabolism. In the chapters on heart and lung disease there are excellent reviews of the physical diagnosis of these systems. There is a thirty page review of electrocardiology.

The illustrations are well selected and well reproduced and the paper and printing are of first quality. The index is good.

Except for the lack of references, the book is excellent. One might take exception to some minor statements (such as the use of Vitamin E in sterility) but, in general, the author has presented an informed, integrated and balanced discussion.

DOUGLAS CARROLL

Modern Treatment. A Guide for General Practice. By Fifty-Three Authors, Edited by AUSTIN SMITH AND PAUL L. WERMER. 1146 pp., $20.00. Paul B. Hoeber, New York.

This is an encyclopedic collection of current medical therapy bringing to mind two other publications of a similar nature which enjoy considerable popularity with the general practitioner. It is a book written in a connected style and considering the number of authors is very uniform in approach. It has a minimum of tabulated treatments and in this respect differs from a very popular annual on treatment but like this annual does not list any references. As far as is possible in a book of this size and scope the treatment mentioned is modern and there is a minimum of worthless old remedies that are so often perpetuated in this type of work. Beside the routine treatment of disease there are to be found references to the philosophical and ethical treatment of a patient.

The book is divided into sections beginning with sections on patient-physician relationship, ethical restrictions, and the general principles of the treatment of infections. Then the book launches out into a description of the treatment of the common and some of the less common diseases. Each treatment is preceded to a varying degree by a description of the disease state and this description is often remarkably full. Later are found sections on nutritional principles, medical treatment in the specialties with particular reference to the problems that are likely to be met with or can be dealt with by the general practitioner, and there are brief discussions on surgical principles, the treatment of shock, the use of anaesthetic and inhalational therapy and the use of physical treatment and radiotherapy. To complete the volume is an excellent section on diagnostic techniques and principles and normal values.

Practical and avoiding vague statements, the book is almost a general practitioner's handbook and although over a thousand pages it is not too cumbersome. The print is clear and the layout is, if anything, too generous. One disadvantage is the price, several medical students and practitioners mentioning that they think it is too high for a book which considering the rapid change in methods of treatment will soon have to be revised.

There are several more specific criticisms of the book. Firstly, there is no list of references and when controversial matters are treated rather dogmatically such a list becomes very important. For example, in the section concerning diseases of the thyroid it is stated that when treating simple colloid goiters in endemic areas by giving iodine, it is possible to cause hyperthyroidism Not everyone would agree with this. Secondly, the giving of over four liters of fluid daily in cases of bronchiectasis could hardly be called standard treatment. The purpose of the fluid is said to be to liquefy the sputum. Then there is no mention made of the use of phenylbutazone in the treatment of arthritis, the use of veratrum in the treatment of hypertension or the use of streptokinase and streptodornase in the treatment of

empyema and although dengue fever is discussed, yellow fever is not mentioned. In the section dealing with asphyxiating gases, under the heading "cyanide" the last paragraph is misplaced as it does not deal with cyanides but with asphyxiation caused by gas and fumes.

All in all, in spite of the rather high price this is a book that can very profitably be read by the general practitioner and referred to for practical methods of treatment at the physician's leisure.

JOHN B. MACGIBBON

Die Kreuzschmerzen der Frau. By HEINRICH MARTIUS. 166 pp., Ganzleinen DM 19.50. Georg Thieme Verlag, Stuttgart, Germany.

The name of Heinrich Martius is held in high esteem by American gynecologists as a most worthy colleague. He is well-known for many previous publications, among them an excellent book on operative gynecology.

This short text on backache in women conforms with the high standards that the author has previously set. Discussions of the anatomy of the pelvis and the back are followed by a resumé of the many gynecological and orthopedic causes of backache. Seventy-three pertinent diagrams and illustrations are most helpful in enriching the text.

The average American gynecologist rightfully believes that most backaches originate in the back rather than the pelvis and may feel that too much stress is laid on the back as an etiological factor. Nevertheless no one will deny the occasional pelvic causation of backache, and this text covers most possibilities in exemplary fashion with appropriate suggestions as to diagnosis and treatment. The urinary tract receives little consideration, and one must conclude that female urology is not the province of the German gynecologist.

There is little doubt that this small volume will have a limited appeal in this country. The reader who is not versed in the German language will, like this reviewer, have to struggle, and the book will be of most value to him who has occasion to talk or write on backache in the woman. Nevertheless any gynecologist or orthopedist will testify as to the importance of this problem, and along with teaching institutions, these specialists may find this a worthwhile addition to the literature.

E. R. NOVAK

The Natural History of Infectious Disease. By SIR MACFARLANE BURNET. 356 pp., $4.50. Cambridge University Press, New York.

The study of medicine and the subject of biology have grown so far apart that students of the two disciplines rarely understand each other and frequently view each other's specialty with suspicion. A few hardy scientists have dared to present their data so that all may understand. Dr. Burnet is foremost among the students of infectious disease in such an attempt.

The first edition of this little volume was published in 1940. It was popular throughout the world and was even a recommended text in several medical school courses. The present volume is thoroughly revised and even takes on a tone of "Just see how slight a threat infectious disease is now"!

Each chapter is a little essay, usually on specific diseases and each has some new facet of the old problems to present. This reviewer found the discussion of the use of bacteriophage in cholera particularly stimulating.

The final chapter is a plea for a continued biological or ecological study of populations and disease, so that the gloomy forebodings of new and more terrible war may be combatted.

"Somehow the use for human domination of nuclear reactions, artificially virulent and artificially disseminated micro-organisms and control of thought and behaviour by unnatural methods, whether pharmacological or psychological must be prevented. Unless this

can be done, the whole biological and social background to the human species is in danger of complete disorganization of a type so apart from any previous experience that nothing less than a new process of evolution will be needed before a healthy civilization becomes possible."

FREDERIK B. BANG

Cybernetics. Transactions of the Ninth Conference, March 20–21, 1952, Edited by HEINZ VON FOERSTER, 184 pp., $4.00. The Josiah Macy, Jr. Foundation, New York.

This volume contains ten papers dealing with a variety of subjects related to communication theory and feed-back mechanisms in biological and social systems. The papers come from a wide range of scientific disciplines; from the pure mathematical to the broadest sociological point of view and include neurophysiology, cellular biology, psychology, anthropology, and psychiatry. The papers were read before a multi-discipline group and following each paper there is a verbatum record of the group's discussion. This is the purpose of the meeting and the publication and constitutes its most interesting and provocative part. The area of the complexity of human communication and especially the position of effect in this behaviour is dealt with more clearly than in any of the preceding conferences.

This book should be of interest to anyone who looks from the point of view of his own specialty to its application to a more general operational understanding of human beings, and hopes to learn from workers in other fields new ways of dealing with, and learning more about, these common problems. There is a bibliography included for each article.

ALLAN R. MCCLARY

Some Conjugated Proteins. A Symposium. Edited by WILLIAM H. COLE. 73 pp., $1.75. Rutgers University Press, New Brunswick, N. J.

This small pamphlet contains the substance of the Ninth Annual Conference on Protein Metabolism held at Rutgers early this year. The constitution, physico-chemical properties, and biological significance of some conjugated proteins are discussed. The specific proteins considered are: hemoglobin (Granick), the cytochromes (Wainio), nucleoproteins (Chargaff), lipoproteins (Oncley), mucoproteins and mucoids (Meyer). In addition there is a section by Seymour Cohen on the metabolism of virus-infected bacteria. The articles are authoritative and concise reviews of current concepts, methods of investigation, and experimental results in the several fields described. Those who seek information on these topics will be well repaid by a reading of this pamphlet.

Worthy of special note are the following introductory comments by one of the participants in the conference: "Scientists, like little fishes, swim in schools. When we open one of our scientific journals these days, we find a very uneven distribution of topics. Some important fields are almost entirely neglected, others seem to explode into bursts of unbelievable mediocrity. Really valuable contributions in the fields most in vogue at present probably are just as scarce as those dealing with the stepchildren of present-day biochemistry. But not all disciplines make it so easy to call each mush a "homogenate," each soup a "partially purified extract," and to speak—when you have nothing whatever— of a "system." There is a real danger that our science may suffocate in its own excrements."

This raises a question. Quis custodiet ipsos custodes?

FRANCIS P. CHINARD

Renal Function. Transactions of the Fourth Conference Oct. 22, 23 and 24, 1952. Edited by STANLEY E. BRADLEY. 189 pp., $3.50. The Josiah Macy, Jr. Foundation, New York.

The present small volume is the fourth in a now familiar series recording the proceedings

of the annual Macy Foundation conference on Renal Function held in October, 1952. The agenda included consideration of "Ion Exchanges Between Extracellular and Intracellular Fluids, Cation Exchanges in the Renal Tubular Epithelium, Ion Transport Across Living Membranes, and Water and Ion Movements Across Intestinal and Renal Epithelium."

The brief, informal, and usually provocative presentations tendered by several well known experts representing varied scientific disciplines are helpfully illustrated by comprehensive diagrams and charts and supplemented by a selected bibliography. The tenor of the ensuing impromptu discussions has been well preserved and the entire text can be followed with facility.

The context of the fourth conference, unlike its predecessors, has been less confined by the connotation of renal function. Indeed, those present seemed concerned primarily with more general aspects of basic water and electrolyte phenomena and their discourse touched on many phylogenetic and organologic entities.

A. H. Owens, Jr.

BOOKS RECEIVED FOR REVIEW

The Anatomy and Surgery of Hernia. By Leo M. Zimmerman and Barry J. Anson. 374 pp., $10.00. The Williams & Wilkins Co., Baltimore, Md.

An Atlas of Pelvic Operations. By Langdon Parsons and Howard Ulfelder. Illustrated by Mildred B. Codding. 231 pp., $18.00. W. B. Saunders Co., Philadelphia, Pa.

Clinical Management of Behavior Disorders in Children. By Harry Bakwin and Ruth Morris Bakwin. 495 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

Clinical Orthopaedics, No. 2. Edited by Anthony F. DePalma. 247 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

Dermopapiloscopia Clinica, Vol. 1, 221 pp., Vol. 2, 291 pp. By Israel Castellanos. Imp. P. Fernandez y Cia., S end C. Hospital numero 619, La Habana, Cuba.

Die Buerteilung der Zurechnungsfähigkeit. By Kurt Schneider. 36 pp., kartoniert DM 2.70 Georg Thieme Verlag, Stuttgart, Germany.

Die Zückerkrankheit. By Ferdinand Bertram. 175 pp., Ganzleinen DM 19.80. Georg Thieme Verlag, Stuttgart, Germany.

Infrared Absorption Spectra of Steroids. An Atlas. By Konrad Dobriner, E. R. Katzenellenbogen and R. Norman Jones. 308 pp., $11.50. Interscience Publishers, New York.

Modern Clinical Psychiatry, 4th edition. By Arthur P. Noyes. 609 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

Peripheral Nerve Injuries. Principles and Diagnosis, 2nd edition. By Webb Haymaker and Barnes Woodhall. 333 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

Respiratory Diseases and Allergy. By Josef S. Smul. 80 pp., $2.75. Medical Library Co., New York.

Shock and Circulatory Homeostatis. Transactions of the Second Conference, Oct. 19, 20, 21, 1952. Edited by Harold D. Green. 275 pp., $3.75. The Josiah Macy, Jr. Foundation, New York.

The Surgery of Infancy and Childhood. Its Principles and Techniques. By Robert E. Gross. 1,000 pp., $16.00. W. B. Saunders Co., Philadelphia, Pa.

Surgery of Trauma. Edited by Warner F. Bowers. With Forewords by Melvin A. Casberg and Surgeons General of the Army, the Navy and the Air Force. 605 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

Sympathetic Control of Human Blood Vessels. By H. Barcroft and H. J. C. Swan. 165 pp., $3.75. The Williams & Wilkins Co., Baltimore, Md.

Sympathikus Chirurgie. By Paul Sunder-Plassmann. 162 pp., Ganzleinen DM 54. Georg Thieme Verlag, Stuttgart, Germany.